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PHYSICAL CHEMISTRY OF VITAL PHENOMENA

FOR
STUDENTS AND INVESTIGATORS IN THE
BIOLOGICAL AND MEDICAL
SCIENCES

BY
J. F. McCLENDON
Assistant Professor of Physiology in the University of Minnesota

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TO

EDWIN G. CONKLIN

AND

HERBERT S. JENNINGS

WHO ENCOURAGED MY EARLY EXPERIMENTATION

42944

PREFACE

This book comprises a course of lectures and laboratory work given to graduate and advanced medical students in the University of Minnesota. The need for special teaching in the application of the simpler aspects of physical chemistry to biology was first realized by the author while attempting to teach physics and physiology to the same group of college students in 1906. A collection of abstracts of papers on the subject was commenced but interrupted by various affairs, especially the change to medical teaching. It became evident, however, that even medical students with a crowded curriculum would do well to devote any available time to this subject if they expect to do any research or read current medical literature.

The purpose of the book is not to go far into physical chemistry but to develop a tool for physiological research. Lengthy discussions of debated questions are avoided by tentatively accepting the hypothesis which fits the most facts, until a better one appears. For further discussion of any subject the reader is referred to the literature list and index. For facts, however, he is referred to nature. It is not to be hoped that theories should coincide exactly with data available at present. Even in the most exact branches of chemistry the atomic weight determinations, for instance, do not exactly coincide with the values calculated from the atomic numbers, and there seems to be some doubt as to whether lead is one element or several. How much more uncertainty there should be about physiology, where determinations are vitiated by the great variability of the material and its physiological states.

The literature list was compiled from abstracts made in various libraries during the last ten years in addition to published abstracts and summaries. In some cases only part of a paper or the summary was read. Following the date is the gist of some data to which it is desired to call attention, or some conclusion which the data apparently justify but which may not be accepted by the author of the original paper. The index was made on the basis of these brief notes and hence cannot be relied on to indicate all the papers on a subject that appear in the literature list. The appendix is for the convenience of a limited class of readers.

The author expresses his sincere thanks to Professors F. E. Bartell, E. G. Conklin, I. H. Derby, G. A. Hulett, A. F. Kovarik, E. P. Lyon and others for examining the copy or parts of it and offering suggestions, and to Margaret S. McClendon for assistance in its revision.

J. F. McClendon.

Minneapolis, October 1, 1916.

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CHAPTER I

INTRODUCTION

The chemist who turns his attention to biological problems meets at the start a seemingly insurmountable barrier. All living matter being composed of cells, and the surface of the cell in such an unstable condition that it is changed by very mild physical or chemical treatment, the rough treatment necessary for chemical analysis is out of the question. This surface layer of the protoplasm has been called by physiologists the plasma membrane. If this plasma membrane is destroyed the entire protoplasm undergoes rapid changes (cytolysis). The protoplasm, therefore, is excluded from the ordinary chemical methods of investigation, the methods that may be applied to the interior of living cells being at present very few, and concerned chiefly with the inorganic constituents.

Modern biochemistry is therefore not yet concerned directly with the composition of normal living cells, but with their decomposition products and the exchange between the cell and its surroundings. While the entire cell is treated as a unit and sometimes called protoplasm or the living substance, we have many reasons to believe that it is composed of a large number of different chemical substances, some distributed throughout the cell and others confined to certain regions, some in solution and others in the form of jellies (gels) or solids. The living cell shows a visible structure under the microscope, and chemical differences between parts of the structure may be detected after death of the cell.

From our knowledge of the decomposition products of cells and the exchange with the medium, we may speculate on the composition of the cell and the changes that go on in it during functional activity. But before doing this the most exact quantitative data on the exchange with the environment are desirable. It is the purpose of this introduction to show that these data cannot be obtained or interpreted without the aid of the methods of physical chemistry. Quantitative chemistry is based on the molecular and atomic theories, and since molecules and atoms obey physical laws, physical chemistry is necessary in order to understand the reactions that take place between them. Though the problems considered in this book are physiological, the methods of attack are chiefly those of the physical chemist.

As an illustration of the usefulness of physical chemistry we may consider the grouping of the elements into the periodic system. Fig. 1 shows the elements grouped according to their physical characters. It may be seen that Li, Na, K, Rb and Cs have corresponding positions in the curves. They also have similar effects physiologically. Ca, Sr and Ba form another series, whereas Mg stands somewhat apart physiologically as well as physically, and the same is probably true of Be (near B). F, Cl, Br and I form another series (F, Cl and Br are indicated by spaces homologous with I).

It is sometimes supposed that very exact data are not necessary in biochemistry owing to the errors in taking samples and the individual variation of the organisms. We might be interested, for instance, in determining whether dextrose were burned in an animal, but consider the exact rate of oxidation to be of secondary importance. In reality, all qualitative distinctions are based on quantitative differences. When we say that coal does not burn in air at room temperature we mean that the combustion is very slow. Furthermore, the rate of a chemical reaction may determine the possibility of finally detecting the end products.

All chemical reactions are theoretically reversible. That is to say, not only does the observed reaction take place, but the opposite reaction is taking place at the same time at a slower rate. The most rapid reactions occur between electrically charged atoms or complexes called ions. Positive ions, called cations, are attracted to and combine with negatively charged ions, anions. If we mix an acid (containing H ions) with a base (containing OH ions) the $H^{\cdot}$ and OH' combine to form H_2O. But the reverse reaction also takes place. Water dissociates into H and OH ions, but their number is so small that in all ordinary chemical work they are never detected. The reason for this lies in the difference in speed of the two reactions. The combination

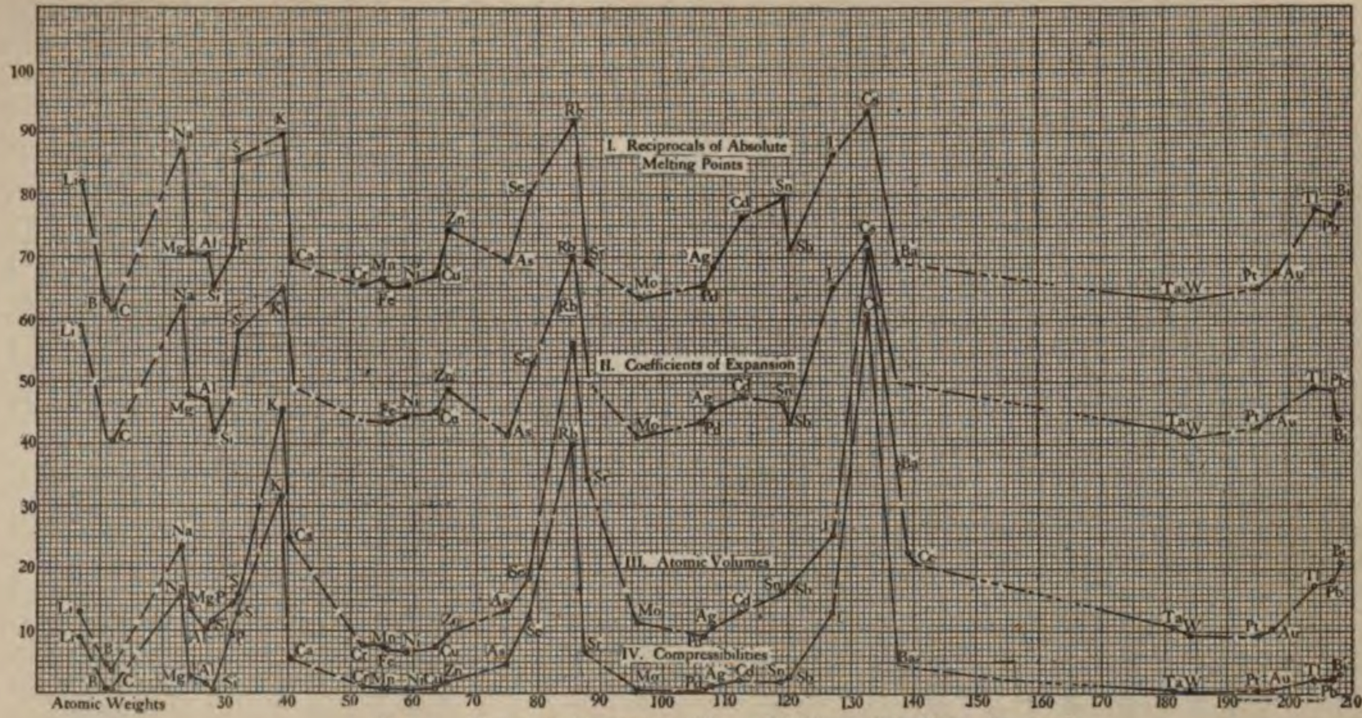


FIG. 1. The periodic system (from JACS, T. W. Richards, 1915).

takes place with enormous rapidity and with the evolution of heat, whereas the dissociation takes place slowly. As soon as a molecule of water dissociates, its ions recombine to form water. This combination takes place whenever a hydrogen ion meets a hydroxyl ion. Hence, the speed of this reaction obeys physical laws.

Since ions of opposite charge combine when they meet, the speed of the reaction depends on the rapidity of movement of the ions and their number in unit volume, or concentration. Since the ionic speed of the same ion at constant temperature and viscosity of the solution is constant, the rate of reaction depends on the concentration. This is called the law of mass action. The behavior of the ions may be compared to men and women in a cotillion. Each man has a chance of meeting each woman. Similarly, each cation has a chance of meeting each anion, and the number of chances of a cation meeting an anion is the number of cations multiplied by the number of anions, in unit volume.

Since the concentration of the reacting substances changes during the reaction, the rate of reaction changes, and is usually negatively accelerated. The mathematical process by which the rate at any particular moment is determined is called differentiation (differential calculus). The rate is determined by the amount of substance transformed, divided by the time. If the rate were uniform we could find it by dividing the difference in the amount of substance at the beginning and end of an observation period, by the difference in time at the beginning and end of the period. But in an accelerated reaction the rate is changing from moment to moment, and the rate at any particular time is expressed by the differential, $\frac{dx}{dt}$. Since the rate cannot be measured in a moment, it is necessary to use the differential calculus in order to estimate it. For our purposes, however, it is not necessary to go through all of the mathematical reasoning, but merely to use the formula supplied by the mathematician, which varies according to the conditions of the reaction.

In actual practice, ionic reactions are usually too rapid to be measured. Even the point of equilibrium may sometimes be so near one end as to be ascertained with difficulty. The above reactions were given as an example because their nature seems to be better understood. The reactions whose rate can be easily measured are usually splittings and combinations of molecules. Many of these have been found to obey the laws of mass action. A monomolecular reaction in unit volume is expressed: $\frac{dx}{dt} = c(a-x)$, where *a* is the original amount of substance, *x* is the amount transformed in the time *t*, and *c* is the constant of the reaction that it is desired to find. From the calculus we obtain: $c = \frac{1}{t} \log_e \frac{1}{a-x}$. In this equation $\log_e$ signifies the natural logarithm (to the base $e = 2.71828$), but this may be reduced to the common logarithm (to the base 10), abbreviated, $\log_{10}$ or log. Since $\log_{10} = \log_e \times .4343$, the above formula becomes: $c = \frac{1}{t}\ .4343 \log_{10} \frac{1}{a-x}$. When several kinds of molecules are concerned the formulae become much more complicated, but in biochemistry we are less often concerned with the rate of reaction than with the point of equilibrium of a reversible reaction.

This equilibrium point in a reversible reaction is illustrated in the case of H_2O. The H_2O dissociates into $H^{\cdot}$ and OH' and these recombine to form H_2O. This is in one direction a bimolecular (bi-ionic) reaction, and in the reverse direction a monomolecular reaction. But this latter, the dissociation of H_2O, may be disregarded mathematically because the concentration of the H_2O (at constant temperature) does not measurably change. At the equilibrium point $[H^{\cdot}] \times [OH'] =$ a constant, where [ ] denotes the concentration of what follows. At 22°, $[H^{\cdot}] \times [OH'] = 10^{-14}$ and therefore if we increase the concentration of $H^{\cdot}$ by adding acid the concentration of OH' is decreased, and vice versa. In pure water $[H^{\cdot}]$ or $[OH'] = 10^{-7}$ at 22° and any variation from this is acid or alkaline. We may measure acidity or alkalinity either by estimating the concentration of the $H^{\cdot}$ or OH', but the hydrogen ions are the ones usually estimated, as will be described in a succeeding chapter.

In any neutral dilute aqueous solution the concentration of the hydrogen and hydroxyl ions is nearly the same as in pure water. A nearly neutral reaction is very necessary to the life of protoplasm. The fluids that bathe the majority of body cells, and the waters in which aquatic organisms live are very nearly neutral in reaction, and are maintained in this condition by the presence of carbonates and phosphates (buffers), as will be described later.

The decomposition products of cells are proteins, carbohydrates, fats and fatlike bodies and neutral salts, as well as other substances that occur in small amount or which may arise from the decomposition of the above. The proteins are composed of amino acids. Amino acids have the general formula NH_2.... COOH, in which the COOH group has acid and the NH_2 group (after hydration) has alkaline properties, so that the amino acid may be nearly neutral. The proteins are formed by the union of the COOH group of one amino acid with the NH_2 group of another with the elimination of one molecule of water. The number of amino acid molecules in one molecule of protein is very large and hence the molecular weight of proteins is enormous and is not very definitely settled in many cases. Before the proteins of the food are taken into the cells they are broken down into amino acids. Each cell builds up its characteristic proteins, irrespective of the proteins given as food.

The carbohydrates are aldehydes or ketones of polyhydric alcohols. Because of their aldehyde character, they are more easily burned in the body, and furnish most of the energy for muscular work. Some of them undergo a molecular rearrangement, giving rise to the lactone form without free aldehyde or ketone groups, but these lactone forms are changed back to the original type in digestion or metabolism. The carbohydrates are stored in insoluble form, and transported in soluble form (sugar).

The fats are esters (salts) of glycerine or other polyhydric alcohols, and fatty acids. The fats in adipose tissue are saturated and are insoluble in water. The fats of other cells are to a large extent unsaturated, and many of them contain a choline and phosphoric acid radical, which makes them capable of forming a colloidal solution with water. These phosphorized fats are perhaps more correctly called phospholipines, but together with cholesterine (a polyhydric alcohol) they have been called lipoids by many physiologists.

The burning of 1 g monosaccharide yields 3.74 large calories, disaccharide 3.95, animal or vegetable fat 9.5, animal protein 5.7. All of this heat is produced by their burning in the body, except in case of protein, which produces only 4.5 calories as it is not completely burned, being eliminated as urea and uric acid and ammonia, etc.

The mineral constituents are apparently for the most part dissolved in the water contained in the cell and will be considered later in regard to their effect on the solubilities (aggregation states) of the proteins and phospholipines.

Among those constituents occurring only in traces the most important are the enzymes, thermolabile substances of unknown composition which accelerate certain chemical reactions. Some of the enzymes are poured out of the cells (as pepsin, for instance) so that they may be easily obtained, but many enzymes that are supposed to exist in all cells have been looked for in vain. Such are the enzymes that are supposed to accelerate the oxidation of the foodstuffs. It now appears that such oxidations are inseparably connected with the cell structure or to some substance that is destroyed when the cell structure is destroyed. The enzymes are greatly influenced in their activity by the hydrogen ion concentration. They may be rendered inactive by adsorption, or by anesthetics and they are influenced to some extent by neutral salts.

Most of the constituents of cells are called colloids on account of their physical properties. Colloids are divided into two classes, suspensoids and emulsoids. Suspensoids are merely solid particles electrically charged and suspended in water, only distinguished from suspensions by the small size of the particles, which cannot usually be seen with the ordinary microscope but only with the ultramicroscope with a powerful dark ground illumination. The suspensoids are precipitated by small quantities of neutral salts or other electrolytes (substances which dissociate into ions), because ions of opposite charge to the particles collide with them and neutralize their charge, the particles gravitating, no longer having the electric charges to hold them up.

Emulsoids are very different in some ways. The colloidal particles partly dissolve. The remainder absorbs water and swells, becoming viscid and the result is an ultramicroscopic emulsion of this viscous fluid. When two substances or fluids do not mix homogeneously they are called phases. In the suspensoid solution one phase is the solid particles and the other the water. In the emulsoid solution both phases contain water and colloid, but one is very rich in water and the other is relatively poor in water. Emulsoids may be precipitated with large quantities of salts in the same way that alcohol may be salted out of water, but they are not affected by the small amount of salt necessary to precipitate suspensoids. The particles of typical emulsoids cannot always be seen with the ultramicroscope, but the particles of lecithin, a phospholipine, for instance, are usually distinct. Many protein solutions which are typical emulsoids are transformed into suspensoids by boiling. This change, which is called denaturation, is caused by many substances, which are therefore used in tests for proteins. Emulsoids increase the viscosity and decrease the surface tension of water, whereas suspensoids do not. Since all substances that reduce surface tension become more concentrated in the surface film, the same is true of the emulsoids. The emulsoid in the surface film gradually changes into an elastic membrane, a so-called haptogen membrane.

Colloids have the peculiar property of forming jellies, called gels to distinguish them from their solutions called sols. The gel formed by a suspensoid consists of a spongelike structure formed of rows of colloidal particles. No structure can be seen in the gels of some emulsoids, but there is evidence for such a structure in their elasticity. An emulsoid increases the viscosity of water. The greater the concentration of the emulsoid the greater the viscosity, until very concentrated solutions have the viscosity of jelly, and are therefore gels. The viscosity of emulsoid sols and gels is not entirely dependent on concentration but is influenced by temperature and the presence of dissolved substances.

Whereas we may learn a great deal about the exchange between the cells and the exterior by studying the intake and output of the lungs, the products of digestion that are absorbed by the alimentary canal and the output of the kidneys, many cells are so far removed from these organs that much is left to be learned about their exchange. These cells are bathed by tissue juice, which is probably somewhat similar to the blood plasma.

In fact, the blood plasma itself comes in contact with some of the cells.

The blood plasma contains amino acids, sugar and fats for the nourishment of the cells. The neutral salts in the plasma maintain the proper viscosity of the emulsoids, especially those of the plasma membranes of the cells. The carbonates, phosphates and to a small extent the proteins maintain the nearly neutral reaction of the plasma. The chief function of the serum albumin and globulin is not known; these proteins increase the viscosity of the blood and throw more work on the heart in maintaining the circulation. They are probably not absorbed by the cells because they appear to be different from the cell proteins. They probably exert a protective action on the plasma membranes of the cells and increase the solubility of certain substances, such as uric acid, by holding them in colloidal solution. Many intermediate products of metabolism and waste products are carried from certain cells to certain others by the blood. The presence of hormones in the blood, which stimulate certain organs to activity, seems to be established, but the chemistry of these substances, with the exception of adrenalin, is unknown. The plasma also contains the substances that form the fibrin of blood clots, and many enzymes whose function is not very clear.

The exchange between the cells and the fluid bathing them is caused by diffusion of dissolved substances, but if this were unlimited the dissolved substances of all of the cells would be the same, which is not the case. Diffusion is fairly rapid through water but decreases as the viscosity increases. The increase of viscosity due to emulsoids does not appear to explain entirely the limitation of the diffusion of some substances, and the problem has never quite been solved, though many models showing how it might occur have been borrowed from pure chemistry. Traube's membranes form one series of models. If a solution of potassium ferrocyanide is brought in contact with a solution of a copper salt, a film of copper ferrocyanide is formed at the plane of contact of the two liquids. This film is easily permeable to water but impermeable to potassium ferrocyanide, copper salts and many other substances, such as sugar. The permeability of this membrane may be increased by treating it with certain solutions without destroying its mechanical continuity. During this process the membrane seems to change from a colloid structure to a mass of microscopic crystals.

We do not know the nature of the plasma membrane, but the normal cell is impermeable, or very poorly permeable, to certain salts and some other substances. Some fish eggs are impermeable to salts and to water. The permeability of cells is increased on stimulation, and goes back to normal during rest. All cells are permeable to a host of substances that reduce the surface tension of water to a marked degree, and to many substances that dissolve in certain non-aqueous media, although some substances that do not reduce the surface tension of water or dissolve in these media penetrate cells.

If any solution is separated from pure water or a less concentrated solution by a membrane to which the dissolved substance (solute) is impermeable, the solute exerts pressure on the membrane, and the water passes through the membrane into the solution. This pressure is called osmotic pressure, and depends not on the percentage of solute, but on the number of molecules and ions per unit volume. The standard concentration is the molecular weight of a substance in grams dissolved in a liter of water. This exerts an osmotic pressure of 22.4 atmospheres and is called a mol. If the molecules partly dissociate into ions the osmotic pressure is increased. The osmotic pressure is difficult to measure directly because it is difficult to obtain membranes that can stand the pressure. Since it has been found that the osmotic pressure is proportional to the lowering of the freezing point of pure water due to adding the dissolved substance, the freezing point is usually determined and the osmotic pressure calculated. When the osmotic pressure is greater on the inside than on the outside of a cell, the cell expands unless the cell wall is strong enough to stand the full pressure without stretching. In green plants the osmotic pressure is greater on the inside than in the water bathing the roots, because the dissolved substances are mostly manufactured on the inside. Plants use osmotic pressure as the force for growth, and can force asunder great rocks when they grow in a crack between them. The osmotic pressure of the blood of mammals is almost as constant as the body temperature, but we do not thoroughly understand its role in physiology. When an organ works, large molecules are broken down into small ones, the number of molecules and the osmotic pressure are increased and water is drawn out of the blood. But when the organ rests, or grows, and large molecules are formed of small ones, the reverse process does not occur. At least the water does not flow in the opposite direction. The water that was drawn out of the blood in the first instance finally passes into the lymph vessels and then back into the blood, causing an increased flow of lymph, but the lymph flow never changes in direction.

The restricted permeability (called semipermeability) of the plasma membrane of muscle and nerve cells gives the clue for understanding the electric phenomena of these tissues. The resting plasma membrane seems to be permeable to some cations but impermeable to all anions. The cations of some substances that are more concentrated within the cell than outside diffuse through the plasma membrane, but they cannot go far, being held back by the attraction of the opposite electric charge of the anions. These cations on the outer surface of the plasma membrane give the whole cell surface a positive charge. If the plasma membrane is stimulated at one point, this altered region becomes permeable to anions and therefore negative in comparison with the unaltered portion. If the altered and unaltered portions are connected by means of a wire, a current flows through the wire from the unaltered to the altered region. This electric current is called the action current or the current of injury, according to whether it is caused by stimulation or injury of the plasma membrane. Such bioelectric currents are very strong in the electric organs of certain fish, are also noticeable in the sensitive plants and in glands, and perhaps occur generally.

Since the body is made up of numerous cells and each cell is composed of several phases, the surface phenomena are very important. While the interiors of solutions are homogeneous, the surface film has peculiar properties, due to the strained relations of the molecules. Dissolved substances (solutes) that reduce the surface tension of the solvent tend to become more concentrated in the surface film, whereas solutes that increase the surface tension are less concentrated in the surface film. If a solute that decreases the surface tension of one phase, becoming more concentrated in its surface film, is soluble in the second phase, it diffuses into the second phase (is *absorbed* by it); but if it is insoluble in the second phase it remains concentrated in the phase boundary, and is said to be *adsorbed* by the second phase.

Electric polarization, such as was described above in regard to nerve and muscle, decreases the surface tension. Hence stimulation is followed by local increase in surface tension. Surface tension changes cause ameboid movements and cell division. Some suppose that muscular contraction is due to surface tension changes, but if this is the case the surfaces concerned must be those of colloidal particles or internal structures, as the surface of the muscle fiber is not large enough to account for the force of contraction.

We thus see that a knowledge of what is ordinarily called chemistry is not sufficient for the biochemist dealing with medical or biological problems. Beside the reactions between proteins, carbohydrates, fats, and many organic and inorganic compounds as usually considered by the biochemist, we are concerned with such physico-chemical processes as the rate of reaction and the position of equilibrium as expressed in the law of mass action, with the effect of ions, especially $H^{\cdot}$ and OH', osmotic pressure, phase boundaries and the surface tension, diffusion, adsorption and electrical polarization phenomena that occur at the phase boundaries, with colloids and their aggregation states, whether gels or sols, with the effect of salts on these colloids, and especially on the plasma membrane which is apparently a colloidal structure, with the enzymes and their colloidal state and dissociation, and finally with certain reactions that are apparently accelerated by cell structure, presumably through the intermediation of adsorption phenomena.

Definitions of Physical Properties of Aqueous Solutions

There is a group of properties of water which are affected proportionately by the introduction of a solute. These are called by Washburn, 1915, the colligative properties of the solution. If the solution is sufficiently dilute, the effect of the solute on its colligative properties is independent of the chemical nature of the solute, and determined solely by the number of dissolved particles (molecules + ions). The colligative properties are:

1—Vapor Pressure. If water is introduced into a vacuum it will evaporate until the pressure of the vapor reaches a certain value, dependent on the temperature. The vapor pressure of pure water at 20° is 17.4 mm, at 25° 23.5 mm, at 30° 31.6 mm, and at 37° 46.7 mm. The lowering of the vapor pressure by 1 mol solute at body temperature is only about .5 mm and is less at room temperature since it is a relative lowering and the vapor pressure of pure water is less at lower temperature.

2—Boiling Point. The boiling point of water is raised .54° by 1 mol solute. (Vol. = 1 liter where not stated.)

3—Freezing Point. The freezing point is lowered 1.85° by 1 mol solute.

4—Osmotic Pressure. If a mol solution is enclosed in a vessel whose walls are impermeable to the solute and permeable to the water, and immersed in pure water, water will pass into the vessel, causing a pressure, osmotic pressure, of 22.4 atmospheres.

With these colligative properties might be classed electrode potentials, since they depend on osmotic pressure. The concentration of a solute calculated from its effect on all colligative properties is the same, but may differ from its concentration calculated from other data, so the word *activity* is sometimes used to denote concentration calculated from colligative data.

The density of the solution is affected by the concentration of the solute, but the degree of change differs with different solutes. Hence, this property of a mixed solution cannot be interpreted unless the proportion of the different solutes remains approximately the same (as in sea water, for instance). Density is determined by weighing a certain volume of the solution measured in a pycnometer and dividing its weight by the weight of an equal volume of water. The rule has been to have the water at 4° and the solution at 15°, but in many determinations the water is taken at the same temperature as the solution, and 25° is rapidly becoming the standard temperature for all sorts of measurements.

The refractive index is also used to determine concentration. With the Abbe direct reading refractometer only a drop of the solution is necessary, and hence this instrument is used in protein analysis when the protein is pure or the accompanying solutes are known. The Zeiss interferometer gives a more accurate reading but requires a larger sample. One per cent of protein raises the refractive index .001 per cent.

The viscosity of the solution is affected very little by neutral salts but very much by emulsion colloids. Not only does the concentration of the emulsoid change the viscosity, but (at the same concentration) the colloidal state affects it. Apparently, the greater the hydration of the colloid the greater the viscosity. The viscosity of a solution is measured by the time required for it to flow through a capillary tube divided by the time required by pure water to flow through the tube at the same temperature.

The rotation of the plane of polarized light may be used to find the concentration of an asymmetric solute in a mixed solution since only molecules having an asymmetric atom, usually carbon, have this property. Rotation is measured with a polarimeter. Colloidal solutions may polarize light dispersed by the particles (Tyndall effect) and gels polarize light when deformed, and are then called doubly refractive or anisotropic.

The surface tension may be used as an index of the concentration of certain solutes. Neutral inorganic salts have very little effect on the surface tension, but anesthetics have a marked effect. The surface tension is found by dividing the weight of a drop of the solution by the weight of a drop of water dropped from the same pipette.

The electrical conductivity is much used to find the concentration of ions, but it depends, not only on the number, but also on the speed of the ions, and hence on the viscosity of the solution. The greater the hydration of an ion the less is its speed, hence the speed is supposed by some writers to decrease with decrease in concentration until maximal hydration occurs. When such a dilution is reached, further dilution evolves no heat, and the solution is called "dilute."

The color of a solution is determined qualitatively with the spectroscope and quantitatively with the colorimeter. A change in the quality of the color is supposed by Stieglitz to be due to change in the positions of electrons within the molecule, following an upsetting of the equilibrium by addition or removal of atomic groups. It seems probable that a change in the degree of hydration may lead to a change in color. The color of a sol may be due to the size and refractivity of the colloid particles.

The degree of turbidity of a sol or suspension is determined with Richards's nephelometer.

In order to facilitate comparison of the results of different investigators, the standards used in measurement should always be recorded. The practice of measuring dimensions in the metric system and temperature in degrees centigrade (Celsius) is quite general, but some confusion may arise from any assumptions as to the concentration of solutions. The effect of the acceleration of gravity may be neglected in weighing, because it affects equally the weights used and the substance weighed. The buoyancy of the air is taken into consideration in the finest work and especially for very light substances. Certificates of the U. S. Bureau of Standards give weights in vacuo, hence the volume of the weights must be subtracted from the volume of the substance weighed in correcting for the buoyancy of the air.

The lengths of the two arms of the balance beam are often sufficiently different to produce an appreciable error in weighing. By determining the ratio of the lengths of the two arms a correction may be applied. Or the true weight may be found by taking the geometric mean of the weights on the two pans (after a second weighing on the opposite pan).

The standard solution or *m*, is the gram-molecular-weight of the solute in a liter of the solution, but the liter flask from the Bureau of Standards should be used at the temperature for which it was standardized. After the solution is standardized, any change in temperature changes its volume and hence burettes and pipettes must be used at the same temperature as the flask. A flask standardized at 15° would hold 0.4 cc too much at 30°, but if it is merely a question of volumetric analysis, this can easily be corrected for as the pipettes and burettes likewise hold 0.04 per cent too much. If a liter flask is standardized at 15° it holds about 998 g H_2O at 15°, 996.3 at 25° and 995 at 30° when weighed in air. This refers to the true liter (the volume of a kilogram of H_2O at 4°) and not to the Mohr liter. A liter measures 1000.027 cc but in this book cc is used to indicate 0.001 liter or milli-liter (ml).

It is often desirable to use solutions under various conditions, and since their concentration according to the above definition may change, new definitions are substituted. Since the molecular weight of a substance may change upon solution, the weight in grams as given in the chemical formula (mol) is usually taken, and the solution of this in a liter is called *formal* but is designated here as mol in order to save space. When attention is especially directed to an element that occurs more than once in the formula, the molecular weight is divided by the number of times this substance occurs in the molecule (= gram equivalent weight), and the solution of this in 1 liter is then called ***normal.*** The effects of temperature are eliminated by using *weight normal* solutions, in which the formal or normal (gram equivalent) weight of the substance is dissolved in 1000 grams of the solvent, but such solutions require a new standardization before use in burettes. The requirements of volumetric analysis are satisfied in the method used in titrating the chloride content of sea water. The results are expressed as grams of Cl per kilogram of sea water. The volumetric apparatus is standardized with a bottle of standard sea water at the temperature at which the titrations are to be made. It is then necessary merely to have a relative calibration of the divisions on the burette.

CHAPTER II

ELECTROLYTIC DISSOCIATION

Electrolytic dissociation is not dependent on electrolysis as was formerly supposed. When certain substances are dissolved in water they dissociate into electrically charged ions, and these substances are therefore called electrolytes, to distinguish them from poorly dissociated, so-called non-electrolytes, such as sugar or urea. The individual ions in gases may be detected, but in solutions we are usually content with the circumstantial evidence of their existence. In biology we usually have to deal with aqueous solutions, and these are implied where others are not stated. Electrolytes that dissociate to a great extent in water dissociate much less if at all in many non-aqueous solutions.

Strong acids and bases and their salts are almost completely dissociated in very dilute solutions, the dissociation increasing with dilution. Weak acids and bases and their salts are as a rule poorly dissociated, but salts formed of a weak acid and a strong base or a weak base and a strong acid are more strongly dissociated. As a rule, inorganic acids and bases are strong and organic weak, but there are exceptions.

Solutions of all chlorides ($NaCl$, KCl, $LiCl$, $CaCl_2$ . . .) contain chlorine ions, $NaCl$ dissociating into positively charged Na ions ($Na^{\cdot}$) and negatively charged Cl ions (Cl'). These ions are never free as represented but always hydrated. Silver nitrate in solution dissociates into $Ag^{\cdot}$ and NO_3'. If silver nitrate is added to the solution of any chloride a white cloud of $AgCl$ is instantly formed because $Ag^{\cdot}$ unites with Cl', the two being drawn together by their electric charges.

Hydrated cobalt ions give a pink color to their solution, but the undissociated salts (and possibly non-hydrated ions if they exist) are of different colors, as may be seen by dissolving them in solvents in which they do not ionize. Cobalt chlorid in alcohol is blue, but if water is added the solution turns pink. Cobalt nitrate in alcohol is purple, but on adding water it turns pink, because in the water-alcohol mixture electrolytic dissociation takes place (Noyes and Blanchard, 1900).

Electric conductivity of solutions: Solutions of electrolytes conduct the electric current and are called conductors of the second class to distinguish them from the metals which are conductors of the first class.

If an electric current is passed through a solution from two metallic conductors (electrodes) the positive ions (cations) are attracted to the negative electrode (cathode) and the negative ions (anions) are attracted to the positive electrode (anode).

The more ions there are in a solution the greater the electric conductivity, and hence the electric conductivity may be used to determine the ionization. In doing this the electrical resistance, in ohms, is measured, and the conductivity is the reciprocal of the resistance.

The electrical resistance of solutions is measured by means of the Kohlrausch method. The solution to be examined is placed in a conductivity cell which consists of a glass vessel containing two platinum electrodes. The electrodes are made of platinum foil and should stand vertically so that bubbles will not collect on them. Every time the distance between the electrodes is changed to the slightest degree the cell has to be standardized again. To avoid this, the platinum is often made thick for stiffness, or the four corners of one electrode are connected to the corners of the other by means of glass rods fused to the platinum by heat. To the upper edge of the platinum foil a stout platinum wire is welded. This is done by heating foil and wire to a white heat and welding the wire to the foil by means of a quick tap with a hammer. The wire is fused into the end of a small glass tube and a drop of mercury dropped into the tube so as to touch the platinum wire. When connecting up the apparatus, a copper wire is inserted down the tube into the mercury. The electrodes may be fastened into the cell by passing the glass tubes through a stopper that fits into the cell.

For solutions used in pure chemistry the pipette forms of conductivity cells that are on the market are very convenient. For biological fluids when only a small quantity may be obtained, the test tube form of cell shown in Fig. 2 is convenient. For tissues, the apparatus shown in Fig. 3 is useful.

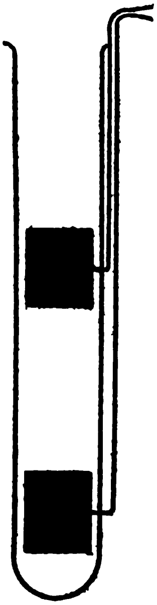


Fig. 2. Electric conductivity electrode of low inductive capacity.

Before using the cell, it must be cleaned with a saturated solution of potassium bichromate in concentrated sulphuric acid (called cleaning fluid). The platinum should be cleaned by heat before fusing in the glass, and cleaned with cleaning fluid in the cell. The cell is rinsed with distilled water and the platinum is coated with platinum black (platinized) in order to increase its

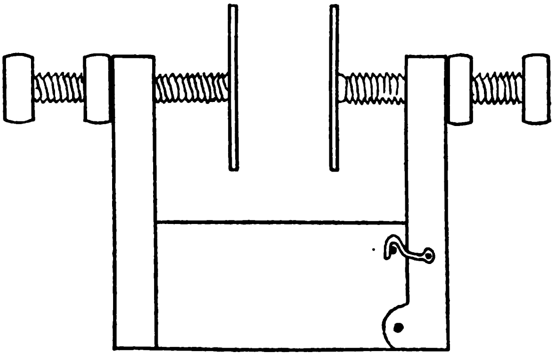


Fig. 3. Electric conductivity electrode for tissues.

surface and reduce electric polarization. In doing this the cell is filled with about 2 per cent platinic chloride solution containing a trace of basic lead acetate. An electric current of about four volts is passed through the cell until one electrode is blackened and the current is reversed until the other pole is blackened. The platinizing solution is returned to the bottle and the cell rinsed and filled with dilute sulphuric acid. A current is now passed in both directions as before. In this way hydrogen is produced, first on one electrode and then on the other, and reduces any chlorine that may have been absorbed by the platinum. The cell is now rinsed many times, finally with conductivity water, is filled with the latter and left until used. Conductivity water is made by adding sulphuric acid and potassium bichromate to distilled water and redistilling it, then adding barium hydrate to it and distilling it the third time with the exclusion of CO_2 of the air. Both distillations may be done at once (Jones, Hulett).

In order to reduce polarization still further, a rapidly alternating current is used. This is best obtained from a special electric generator, or Vreeland oscillator, but owing to its cost most people are content with a small induction coil. The vibrator of the coil should be very stiff or very short, in order to produce a high pitch resembling the sound of a mosquito. A great change in the frequency may change the resistance measured 3 per cent (W. A. Taylor, 1915). The coil should be enclosed in a sound proof box.

A Wheatstone bridge is built up of a resistance box, the con-

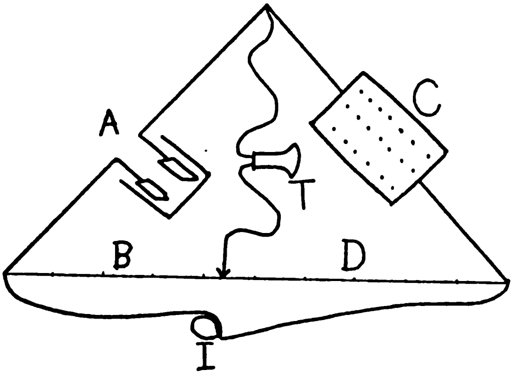


FIG. 4. Wheatstone bridge arrangement for conductivity apparatus.

ductivity vessel, a meter resistance wire with a sliding contact, and a telephone with which to detect the current (Fig. 4). These resistances are connected in a circle. The meter wire is divided by the sliding contact into two resistances which form two legs of the circuit, the box and the cell forming the other two legs. The two secondary poles of the induction coil are connected to the sliding contact and the juncture between coil and box respectively. The conductivity cell is marked A and the adjacent leg of the slide wire B, the box marked C and the adjacent leg of the slide wire D. The current entering the circuit at the juncture of A and C divides, part going through A and B and the other part through C and D. If the two ends of the meter wire have the same electrical potential, then the resistances of $\frac{A}{B} = \frac{C}{D}$, also $\frac{A}{C} = \frac{B}{D}$. In order to determine whether the two ends of the meter wire have the same potential the two wires of the telephone receiver are connected to the two ends and if no sound is heard the two ends have the same potential. If a sound is heard the resistance of the box is changed or the sliding contact on the wire is moved until no sound is heard. The resistance of the box may be read in ohms; the resistances of the two parts of the meter wire are proportional to their length, and hence their lengths are used in the equation. From the equation the resistance of the conductivity cell A may be calculated. The ratio B/D may be read from the accompanying table.

Usually the telephone is never quite silent, and the point of tone minimum may be more or less sharp, but may be sharpened in the following ways. The electrodes are made larger (their surface area increased). A self inductance, which may be varied, is placed in series with A. An electrical condenser, that may be varied, is placed in parallel with C. By readjusting the inductance and condenser and the sliding contact alternately, a sharp tone minimum is finally obtained (Washburn and Bell, 1913). The condenser is to compensate for the capacity of the cell, which is larger the larger and closer together the electrodes are (but is least in the form of cell shown in Fig. 2). The self inductance is to compensate for the self inductance of the box and connecting wires. Resistance coils with very low self in-

OBACH'S WHEATSTONE BRIDGE TABLE OF B/D WHERE B = METER SLIDE FROM 0 TO SLIDING CONTACT AND D = REMAINDER OF SLIDE WIRE.

B	400	410	420	430	440	450	460	470	480	490
0	.6667	.6949	.7241	.7544	.7857	.8182	.8519	.8868	.9231	.9608
1	.6694	.6978	.7271	.7575	.7889	.8215	.8553	.8904	.9268	.9646
2	.6722	.7007	.7301	.7606	.7921	.8248	.8587	.8939	.9305	.9685
3	.6750	.7036	.7331	.7637	.7952	.8282	.8622	.8975	.9342	.9724
4	.6779	.7065	.7361	.7668	.7996	.8315	.8657	.9011	.9380	.9763
5	.6807	.7094	.7391	.7699	.8018	.8314	.8692	.9048	.9417	.9812
6	.6835	.7123	.7422	.7731	.8051	.8382	.8727	.9084	.9455	.9841
7	.6863	.7153	.7452	.7762	.8083	.8416	.8762	.9120	.9493	.9881
8	.6892	.7182	.7483	.7794	.8116	.8450	.8797	.9157	.9531	.9920
9	.6921	.7212	.7513	.7825	.8149	.8484	.8832	.9194	.9569	.9966

In the table following, 1 is understood before the decimal point.

B	500	510	520	530	540	550	560	570	580	590
0	.0000	.0408	.0833	.1277	.1739	.2222	.2727	.3256	.3810	.4390
1	.0040	.0450	.0877	.1322	.1786	.2272	.2779	.3310	.3866	.4450
2	.0080	.0499	.0921	.1368	.1834	.2321	.2831	.3364	.3923	.4510
3	.0121	.0534	.0964	.1413	.1882	.2371	.2883	.3319	.3981	.4570
4	.0161	.0576	.1008	.1459	.1930	.2422	.2936	.3474	.4038	.4631
5	.0202	.0619	.1053	.1505	.1978	.2472	.2989	.3529	.4096	.4691
6	.0243	.0661	.1097	.1552	.2026	.2523	.3041	.3585	.4155	.4752
7	.0284	.0704	.1142	.1598	.2075	.2573	.3095	.3641	.4213	.4814
8	.0325	.0747	.1186	.1645	.2124	.2624	.3148	.3697	.4272	.4876
9	.0367	.0790	.1231	.1692	.2173	.2676	.3202	.3753	.4331	.4938

ductance may be made by bifilar winding or lateral compression of the coil into one plane. A sharp tone minimum may be obtained and the telephone not be sensitive enough to detect it. A telephone that is tuned to the pitch of the induction coil is better, but the tune of the coil may change even though the spring of the vibrator is very stiff. A high frequency generator or Vreeland oscillator should not change in pitch, and is necessary for the greatest accuracy.

Since 1° rise in temperature causes an increase in electric conductivity of about 2.5 per cent, the conductivity cell must be placed in a water bath whose temperature is kept constant or observed very accurately at the moment of the determination.

If the electrodes are 1 cm apart and have 1 sq. cm surface the conductivity of the solution in reciprocal ohms is the specific conductivity. The specific conductivity divided by the concentration (m) is called the molecular conductivity (λ). The usual practice is not to measure the electrodes but to calibrate the cell with a known solution of KCl.

It is found that the molecular conductivity of an electrolyte increases with dilution, or, in other words, the dissociation increases. At infinite dilution the dissociation would be complete, and is practically complete at .0001 mol.

The question may be asked: Where does the electric charge of the ion come from? The study of radium has shown that the molecule contains a store of electricity but the positive charges exactly equal the negative charges and the molecule therefore is electrically neutral. These charges may exist free from molecules or ions and are then called electrons. It seems probable that negative electrons have no weight. A molecule of NaCl may be represented by an atom of Na and an atom of Cl, each with a positive charge, connected by means of two negative electrons. When the molecule dissociates the negative electrons go with the Cl atom and give it an excess of one negative charge, whereas the Na atom has a positive charge.

If an electric current is passed through a solution, anions are deposited on the anode and cations on the cathode. It is necessary to pass 96500 coulombs of electricity (= 1 Faraday) in order to deposit 1 gram equivalent of ions on an electrode. If the ions remain on the electrodes as in electroplating, a gram molecule of the electrolyte is removed from the solution by the passage of 96500 coulombs, for a gram equivalent of anions is deposited on the anode and a gram equivalent of cations on the cathode. In any cross section of the solution, if x gram equivalents of anions is moving in one direction, $1 - x$ gram equivalents of cations is moving in the opposite direction. Supposing that the cations move twice as fast as the anions, if we draw an imaginary cross section half way between the electrodes, twice as many cations will pass going in one direction as anions going in the opposite direction, or ⅔ gram equivalent of cations move to the cathode and ⅓ gram equivalent of anions to the anode. Around the cathode 1 gram equivalent of cations is removed by the electrode and ⅔ is gained by migration, leaving a deficiency of ⅓, whereas ⅓ of anions are lost by migration, or, in other words, ⅓ mol electrolyte is lost. At the anode ⅔ mol electrolyte is lost. The change in concentration of the electrolyte at the electrode gives the transport number of the ion. By following the above reasoning backward, the ionic speed may be calculated (Hittorf).

These values of ionic speed are relative. The actual speed of ions is slow, as is shown by electrolyzing a solution containing colored anions around the cathode, and observing the time necessary for them to reach the anode.

The relative speeds of ions may be more conveniently determined by comparing the molecular conductivities of a series of salts that are completely dissociated. The molecular conductivity of a salt is proportional to the sum of the ionic speeds of anion and cation (v+u). Thus, the ratio of the molecular conductivities of NaCl and KCl is the ratio of the ionic speeds of Na and K.

The relative ionic speeds at 18° according to Kohlrausch (1907) are as follows:

H	318.	OH	174.
Li	33.4	F	46.6
Na	43.5	Cl	65.5
K	64.6	Br	67.
Rb	67.5	I	66.5
Cs	68.	NO_3	61.7
NH_4	64.4	CNS	56.6
Ag	54.3	ClO_3	55
½ Ba	55	½SO_4	68
½ Sr	51	½C_2O_4	63
½ Ca	51		
½ Mg	45		

With organic ions, the longer the carbon chain the less the speed (Bredig, 1894).

HCOO	54.5 at 25°
CH_3COO	40.8
C_2H_5COO	36.5
C_3H_7COO	32.7
C_4H_9COO	30.7
$C_5H_{11}COO$	29.2

In general we may divide electrolytes into two classes, the strong and the weak. Strong electrolytes are greatly dissociated, whereas weak electrolytes are poorly dissociated. So-called non-electrolytes may be to a minute extent dissociated. Water is a non-electrolyte; its ions are the most rapid, but it is poorly dissociated. Whereas the concentration of water is about 55 formal (55 × 18 g per liter), only 10^{-7} mol is dissociated at 22°. [Owing to the partial association of H_2O into (H_2O_2) its concentration is less than 55 mol.]

Strong electrolytes have not yet been brought entirely under the law of mass action. Weak electrolytes obey the law, and therefore their ions must obey it. Take, for example, the dissociation of acetic acid at different dilutions. The equation for this sort of phenomenon was found even before the law of mass action was so widely applied, and is known as Ostwald's dilution law.

If *a* represents the degree of dissociation of such an electrolyte, of which one mol is dissolved in v liters, the concentration of the cations and of the anions is $\frac{a}{v}$, and of the undissociated molecules $\frac{1-a}{v}$. Two reactions are going on at the same time, the dissociation of the molecules into ions and the recombination of the ions to form molecules. The rapidity of dissociation depends on the concentration of the molecules, $\frac{1-a}{v}$, and the recombination on the product of the concentrations of the two classes of ions, $\frac{a}{v} \times \frac{a}{v}$, and at equilibrium, $\frac{a}{v} \times \frac{a}{v} = c\,\frac{1-a}{v}$, where c is a constant, or $\dfrac{\frac{a}{v} \times \frac{a}{v}}{\frac{1-a}{v}} = c$ by division we obtain $\frac{a^2}{v(1-a)} = c$. From this equation it is seen that ionization increases with dilution. Expressed in words, it means that the greater the dilution the farther removed the ions are from one another and the less chance they have of colliding with one another and recombining to form molecules, or the chance of their meeting is equal to the product of the concentration of the anions and the concentration of the cations.

This formula does not hold for the strong electrolytes, and Rudolphi (1895) and van't Hoff have applied the empirical formula, $\frac{a^3}{v(1-a^2)} = c$, to them. One reason that the strong electrolytes do not seem to obey the law of mass action may be that they form different hydrates at different dilutions. Some of these hydrates have been crystallized and others detected in other ways. Not only some molecules, but also all ions are supposed to be in the form of hydrates. Sulphuric acid forms the following hydrates (see Fig. 5): $5H_2SO_4 \cdot H_2O$, $3H_2SO_4 \cdot H_2O$, $H_2SO_4 \cdot H_2O$, $H_2SO_4 \cdot 2H_2O$, $H_2SO_4 \cdot 3H_2O$, $H_2SO_4 \cdot 4H_2O$ and $H_2SO_4 \cdot 12H_2O$, at percentages 96, 94, 85, 79, 65, 58, and 30 of sulphuric acid (C. E. Davis, 1915).

It should be emphasized that strong electrolytes exist chiefly as ions in the dilute solutions with which we have to deal in biochemistry. Take, for example, the neutralization of HCl with KOH. Since HCl, KOH and KCl take no part in the reaction and are practically never present, the reaction becomes $H^{\cdot} + OH' = H_2O$. Cl′ and K˙ are present in the same quantities before and after the reaction takes place. That the reaction is simply the formation of water from its ions is illustrated by the fact that no matter what strong base or acid is substituted for the above (at 20°), 13700 calories of heat are produced for 1 mol of acid and alkali used.

Ionization must be considered in studying the solubility of electrolytes. If we pass hydrochloric acid gas into a saturated solution of NaCl, the salt will partially crystallize out. In a saturated solution of NaCl, the product of the concentrations of Na˙ and Cl′ is a constant, and when we add more Cl′ by the addition of HCl, salt crystallizes out until the product assumes its original value. (This principle is used in the recrystallization of NaCl by the introduction of HCl gas into the saturated solution.)

Some electrolytes dissociate into three or more ions. They dissociate by steps, thus K_2SO_4 dissociates into K˙ and KSO_4' and the latter dissociates into K˙ and SO_4''. In certain cases the first step may be complete and the second be incomplete under ordinary conditions. The dissociation of H_2CO_3 and H_3PO_4 will be considered in a later chapter.

In case one of the ions of an electrolyte is weak and the other strong hydrolysis may occur. K_2CO_3 dissociates into K˙ and KCO_3'. K˙ combines with OH′ of the water and forms KOH while the other ion unites with H˙ of water to form $KHCO_3$. Since the KOH dissociates more OH ions than the bicarbonate produces H ions the reaction is alkaline. The further steps in this hydrolysis will be considered later. Hydrolysis is the union with the ions of water already present. When these are ex-

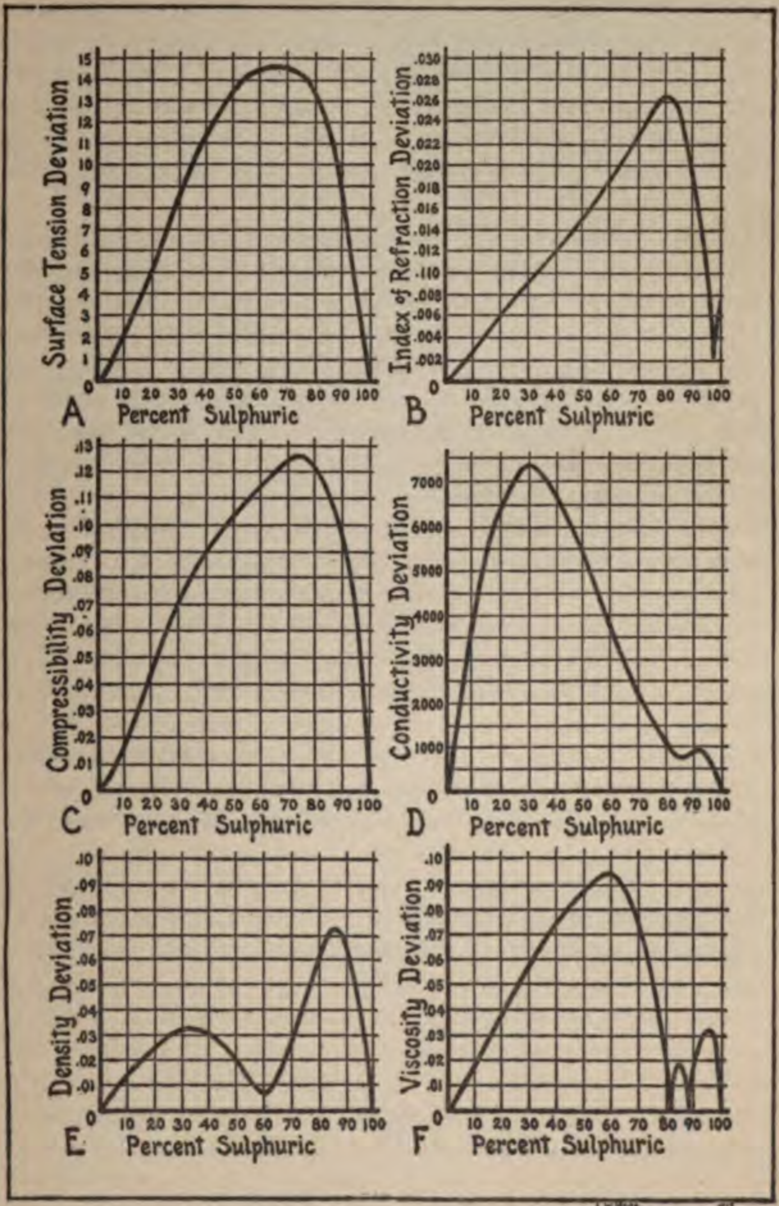


FIG. 5. Curves indicating the concentrations at which changes in the hydration of sulphuric acid occur (from Clarke Edwin Davis, 1915).

hausted more are formed, and the whole process is instantaneous in the above case. In this it is distinguished from the hydrolysis of proteins, for instance, in which the protein is boiled for a long time with acid or alkali, or hydrolyzed by means of enzymes. In the salt hydrolysis water is all that is necessary, but in the case of proteins the hydrolysis occurs with unmeasurable slowness in pure water.

Some substances may dissociate both $H^{\cdot}$ and OH', and are therefore called amphoteric electrolytes or ampholytes. The amino acids and proteins belong to this class. Their general formula is NH_2RCOOH, in which R represents a carbon chain (or a series of them connected by the amino and carboxyl groups). Some of these may be about neutral, but usually the acid or basic character predominates. The double nature of these substances is shown by their compounds. If HCl is added to egg albumin the latter no longer dissociates H ions, owing to the large number already in solution, but it continues to dissociate OH ions and as fast as these are formed their place in the albumin is taken by Cl ions, so that more OH ions continue to form and to combine with the H ions in solution. In this way Alb. Cl is formed, which dissociates into $Alb^{\cdot}$ and Cl'. If an electric current is passed through the solution the albumin will go to the cathode.

On the contrary, if KOH is added to an albumin solution Alb.K will be formed by the displacement of the H of the hydroxyl, and if an electric current is passed the albumin will go to the anode.

At a certain reaction of the solution the albumin will not migrate in an electric field, or if it does half will go toward the cathode and half toward the anode. This reaction is called the isoelectric point for that albumin. This subject will be continued in Chapter IV.

CHAPTER III

OSMOTIC PRESSURE

There are some membranes that are freely permeable to water but impermeable to many dissolved substances, and are called semipermeable membranes. An example of such is found in one of Traube's (1867) membranes. If a solution of potassium ferrocyanide is allowed to come in contact with a solution of $CuSO_4$, a membrane of copper ferrocyanide is formed, which is impermeable to potassium ferrocyanide, $CuSO_4$ and their ions and to a certain degree to some other electrolytes such as $MgSO_4$ and its ions. If this membrane is made in the form of a bag with $CuSO_4$ on the inside and placed in pure water, so much pressure will be developed within it that it will burst. This pressure is called the osmotic pressure of the $CuSO_4$ solution and can be measured if the membrane is strong enough. Membranes may be deposited in the pores of strong clay cups and thickened by driving in the mother substances with an electric current (H. N. Morse, 1914). If such a cup is connected with a mercury or nitrogen manometer so that the osmotic pressure can be measured it is called an osmometer.

It was found by de Vries (1884), Pfeffer (1877) and others that 1 gram molecule (mol) of a non-electrolyte, such as dextrose, dissolved in water to make 1 liter has an osmotic pressure of 22.4 atmospheres at 0°. De Vries found that electrolytes departed from this rule, but Arrhenius (1887) showed that this discrepancy could be explained by his theory of electrolytic dissociation, since the ions exert osmotic pressure as well as the molecules, and hence ionization would increase osmotic pressure as the total osmotic pressure is the sum of the partial pressure of molecules + ions. Van't Hoff (1887) pointed out that osmotic pressure of ideal dilute solutions is the same quantitatively as gas pressure.

The formula for osmotic pressure of dilute ideal solutions is $pv = RT$, where p is the pressure in atmospheres, v is the vol-

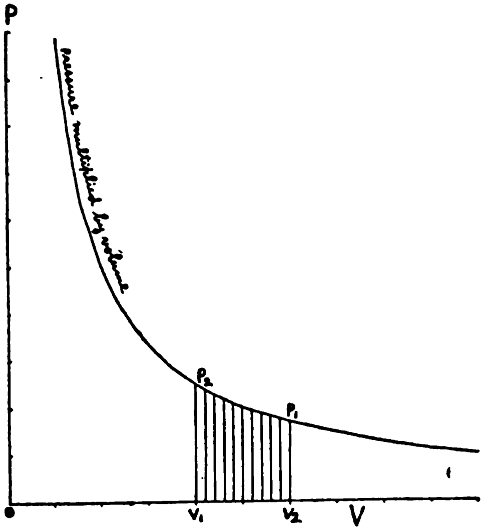


Fig. 6. Graph indicating the method of integrating the formula PV=RT (from MRR).

ume in liters in which 1 mol solute is contained, R = 0.0821, and T is the absolute temperature, in which zero is — 273.090 on the centigrade scale (Fig. 6). Since the volume is the reciprocal of the concentration, the formula becomes $\frac{p}{c} = 0.0821\ T$, where c is the molecular (molar) concentration. If the concentration is 1 and the temperature = 0° C = 273 abs., $p = .0821 \times 273 = 22.4$. In other words, the osmotic pressure of a molar solution is 22.4 atmospheres at 0°. From this a method was developed of determining the extent of dissociation of an electrolyte from its osmotic pressure. If 1 mol KCl is dissolved in so large a volume of water that it is completely dissociated it will exert double the pressure of 1 mol dextrose dissolved in the same volume, because the number of ions is double the number of molecules, and the formula becomes pv = 2RT. If the salt is partly dissociated a number somewhere between 1 and 2 must be used (say 1.5), then 50 per cent of the salt is dissociated.

The most accurate determinations of osmotic pressure have been done on sugars by H. N. Morse (1914) and Berkeley and Hartley (1904). Morse made some determinations on electrolytes, but finds that they increase the permeability of the membrane. The results of Morse do not exactly correspond to the formula, and he supposes this due to the fact that the sugar molecules take up so much room that the concentration of the water is reduced. By dissolving the sugar in 1000 g of water instead of making a liter of solution, the formula was more closely followed. Such solutions are called weight normal solutions, but the water combined with the sugar molecules is not considered. According to Washburn (1915) each sugar molecule is combined with six molecules of water, which means that in a liter of a mol solution, 108 g of water per liter are taken from the solvent. The significance of the room occupied by the molecules is strikingly shown in solutions containing solutes of very high molecular weight. The osmotic pressure of a 30 per cent gum arabic solution is 4 atmospheres, whereas that of a 60 per cent solution is 48 atmospheres. If blood serum whose osmotic pressure is 6 atmospheres is concentrated to ¼ volume, its osmotic pressure is 35 atmospheres.

Owing to the difficulty of determining the osmotic pressure directly, it is usually calculated from the freezing point. An osmotic pressure of 22.4 atmospheres corresponds to a freezing point lowering (Δ) of 1.85°. The following conversion table was calculated by Harris and Gortner (1914) from the formula of Lewis (1908).

In determining the freezing point of aqueous solutions, the only advantage of the Beckmann thermometer is that it may be accessible. It has the disadvantage that it must be adjusted for aqueous solutions and that the zero point must be determined every year. The curvature and the temperature of the two mercury surfaces bordering on the vacuum are not always the same and hence the vapor pressure of the mercury at these two surfaces is not the same, and consequently mercury distils over from one surface to the other. The Heidenhain thermometer has no adjustment to cause this trouble. It is a simple thermometer graduated in hundredths of a degree from about +0.5° to —5°. The Burian-Drucker (1910) thermometer is designed for fluids of which only 1.5 cc may be available. Since it has a smaller bulb and consequently shorter stem, it is graduated in fiftieths of a degree. The thermometer should be kept in ice water several hours before the determination.

TABLE OF OSMOTIC PRESSURES IN ATMOSPHERES FOR DEPRESSION OF THE FREEZING POINT.

Δ	Hundredths of degrees, centigrade									
	0	1	2	3	4	5	6	7	8	9
0.0	0.000	0.121	0.241	0.362	0.482	0.603	0.724	0.844	0.965	1.085
0.1	1.206	1.327	1.447	1.568	1.688	1.809	1.930	2.050	2.171	2.291
0.2	2.412	2.532	2.652	2.772	2.893	3.014	3.134	3.255	3.375	3.496
0.3	3.616	3.737	3.857	3.978	4.098	4.219	4.339	4.459	4.580	4.700
0.4	4.821	4.941	5.062	5.182	5.302	5.423	5.543	5.664	5.784	5.904
0.5	6.025	6.145	6.266	6.386	6.506	6.627	6.747	6.867	6.988	7.108
0.6	7.229	7.349	7.469	7.590	7.710	7.830	7.951	8.071	8.191	8.312
0.7	8.432	8.552	8.672	8.793	8.913	9.033	9.154	9.274	9.394	9.514
0.8	9.635	9.755	9.875	9.995	10.12	10.24	10.36	10.48	10.60	10.72
0.9	10.84	10.96	11.08	11.20	11.32	11.44	11.56	11.68	11.80	11.92
1.0	12.04	12.16	12.28	12.40	12.52	12.64	12.76	12.88	13.00	13.12
1.1	13.24	13.36	13.48	13.60	13.72	13.84	13.96	14.08	14.20	14.32
1.2	14.44	14.56	14.68	14.80	14.92	15.04	15.16	15.28	15.40	15.52
1.3	15.64	15.76	15.88	16.00	16.12	16.24	16.36	16.48	16.60	16.72
1.4	16.84	16.96	17.08	17.20	17.32	17.44	17.56	17.68	17.80	17.92
1.5	18.04	18.16	18.28	18.40	18.52	18.64	18.76	18.88	19.00	19.12
1.6	19.24	19.36	19.48	19.60	19.72	19.84	19.96	20.08	20.20	20.32
1.7	20.44	20.56	20.68	20.80	20.92	21.04	21.16	21.28	21.40	21.52
1.8	21.64	21.76	21.88	22.00	22.12	22.24	22.36	22.48	22.60	22.72
1.9	22.84	22.96	23.08	23.20	23.32	23.44	23.56	23.68	23.80	23.92
2.0	24.04	24.16	24.28	24.40	24.52	24.63	24.75	24.87	24.99	25.11
2.1	25.23	25.35	25.47	25.59	25.71	25.83	25.95	26.07	26.19	26.31
2.2	26.43	26.55	26.67	26.79	26.91	27.03	27.15	27.27	27.39	27.51
2.3	27.63	27.75	27.87	27.99	28.11	28.23	28.34	28.46	28.58	28.70
2.4	28.82	28.94	29.06	29.18	29.30	29.42	29.54	29.66	29.78	29.90
2.5	30.02	30.14	30.26	30.38	30.50	30.62	30.74	30.86	30.98	31.09

The solution to be frozen is poured into a test tube having a double bored cork. This tube is sometimes provided with a side neck through which a small crystal of ice may be dropped to start the freezing as soon as the temperature reaches the freezing point and thus prevent undercooling. The test tube is fitted into a larger test tube and the space between them left full of air or filled with alcohol to prevent the condensation of moisture which would obscure observation. The freezing mixture is usually crushed ice or snow and salt, but this interferes with observation, and ether through which air is sucked is preferable.

The determination of the freezing point is as follows. About 22 cc of the solution is poured into a test tube, and the bulb of the thermometer and a platinum stirrer lowered into it through separate holes in the stopper. The test tube is cooled nearly to the right temperature by immersing it in ice and salt; it is then wiped dry and pushed through the bored cork of a larger test tube and returned to the freezing mixture. The thermometer should not touch the wall of the inner test tube, and this in turn should not touch the outer test tube, as the local cooling thus produced might cause a local freezing before the entire solution reached the freezing point. The solution is stirred until freezing commences. The temperature will fall below the freezing point and when the first ice separates suddenly rise to the freezing point. This undercooling may be lessened by having the freezing mixture only slightly below the freezing point of the solution or by dropping in a crystal of ice to start the freezing at the proper moment. Since the sudden separation of ice that occurs before the definitive freezing point is reached concentrates the remaining solution, the freezing point lowering is too great and should be decreased 1/80 for every degree of undercooling.

Although the cryoscopic method is very simple in principle, it is only by the most painstaking performance that an accuracy of .001° is obtained. Since a Δ of 1.85° corresponds to an osmotic pressure of 22.4 atmospheres, .001° represents a pressure of .0912 atmospheres or 9.2 mm of mercury, and is very serious when small differences in osmotic pressure are to be measured. It should be remembered that the cryoscopic method determines the osmotic pressure at the freezing point of the solution, and this should be reduced to the desired temperature by correcting for the effect of the temperature change on the electrolytic dissociation and osmotic pressure.

A method for determining the freezing point of small amounts of solution, devised by Barger (1904), has been called a microtensimeter, but it is in reality an osmometer in which air forms the semipermeable membrane. It consists of a capillary tube that can be observed under a microscope having an ocular micrometer. The osmotic pressure of the solution is approximately known from previous data or a preliminary test. A graded series of solutions is prepared, the extremes of which will fall on the two sides of the osmotic pressure of the unknown. Drops of the unknown solution alternating with the known solutions are drawn up into the tube and their lengths measured. After an interval of time their lengths are again measured and the drop of unknown that has not changed in length found; its osmotic pressure is between those of the graded solutions on each side of it. One cc of the unknown is sufficient and hence the method is useful in biological work. This method has the advantage that it measures the osmotic pressure at any desired temperature. The chief error is in the mixing of the drops, caused by the adhesion of a surface film to the glass and the transfer of fluid from one drop to another in this film.

The way in which osmotic pressure may do work in the organism is best illustrated in plants. The plant cell is limited by the plasma membrane which is impermeable to some dissolved substances. Surrounding the plasma membrane is the cellulose cell wall. If the cell is placed in water the osmotic pressure of its interior causes it to swell, and the plasma membrane is pressed against the cellulose cell wall and tightens it or makes the cell turgid. This turgidity (turgor) is what gives stiffness to herbaceous plants, lacking which the plant is said to be wilted. The osmotic pressure of many plant cells is a number of atmospheres, and the cell wall has to be very strong in order to withstand the pressure. The extent of this pressure is realized when the roots of trees growing between rocks separate the rocks by the osmotic pressure exerted.

The cell wall of a young plant cell is very thin and is stretched by the osmotic pressure as the cell grows. Some mechanism must be provided for getting the osmotic substance into the cell, before it can stretch the cell and cause growth. In the green plant cell the osmotic substances are manufactured with the aid of sunlight out of H_2O and CO_2, to which the cell is freely permeable. The colorless cells absorb certain organic substances secreted by the green cells, transforming them into other substances to which the plasma membrane is impermeable, and which therefore accumulate in the cells and cause the osmotic pressure. Though these osmotic substances are not in all cases definitely known, tannic and oxalic acid and their salts have been postulated. The sugars are supposed by some investigators to be the nutritive substances passed from the green to the colorless cells, meaning that both kinds of cells are permeable to sugar. Overton, however, claims that plant cells are impermeable to sugar and hence any contained sugar could aid in maintaining the turgor.

A solution whose osmotic pressure is lower than that of the cell is said to be hypotonic, and one whose pressure is greater, hypertonic. The osmotic pressure of many animal cells is very near that of the medium, or the fluid which bathes them. When the medium is hypotonic the cell swells, when hypertonic it shrinks until equilibrium is established. Since animal cells are not surrounded by very strong cell walls, they burst or swell when placed in pure water. But some animal cells are impermeable to water, and consequently are not affected by changes in the medium.

Fresh water protozoa are prevented from bursting by the activity of the contractile vacuole. Zuelzer (1907) acclimatized Ameba verrucosa to sea water and the contractile vacuole beat more and more slowly and finally disappeared in ½ sea water. The few marine protozoa that have contractile vacuole are parasitic and hence not in pure sea water and the pulsations of the vacuoles are much slower than is the case with fresh water forms.

Most of the cells in the mammalian, reptilian and avian bodies are bathed by fluids whose osmotic pressure is practically constant. One of these fluids is the blood. Its osmotic pressure at body temperature is about 8 atmospheres (6.7 at 0°), its freezing point being about —.56°. This corresponds to about 10 per cent (.3 molecular) cane sugar or .95 per cent NaCl solution (.16 mol). See Fig. 30, Chapter XV.

More minute data on osmotic pressure will be considered in Chapter XV.

CHAPTER IV

HYDROGEN AND HYDROXYL ION CONCENTRATION AND CO_2 PRESSURE

Water is an electrolyte and dissociates into H and OH ions, which are therefore equal in number. At 22° or 23° $H^{\bullet} = OH' = 10^{-7}$, or $H^{\bullet} \times OH' = 10^{-14}$. If we increase $H^{\bullet}$ by adding acid, OH' decreases, because $H^{\bullet} \times OH'$ remains equal to 10^{-14}. Conversely, if we add alkali, $H^{\bullet}$ decreases, and hence we may estimate the acidity or alkalinity of a solution by determining the $H^{\bullet}$ concentration. The product of the H and OH ion concentrations is 10^{-14} and the — logarithm is 14 (and is designated — log K_w). The following table gives the values of —logK_w for various temperatures, calculated thermodynamically from the results of Lorenz and Boehi (1909) at 18°, and which agree with those of Sorensen, Michaelis and some others within about one degree.

t°	—logKw	t°	—logKw	t°	—logKw	t°	—logKw	t°	—logKw	t°	—logKw
18	14.142	22	14.004	26	13.861	30	13.733	34	13.611	38	13.513
19	14.108	23	13.960	27	13.828	31	13.702	35	13.581	39	13.475
20	14.073	24	13.927	28	13.796	32	13.671	36	13.561	40	13.446
21	14.038	25	13.894	29	13.765	33	13.641	37	13.532		

In order to save space it is better to express $H^{\bullet}$ concentration as the minus logarithm (PH). Thus if $H^{\bullet}$ concentration $= 10^{-7}$, PH = 7 (Sorensen 1909).

In order to understand the method by which the H ion concentration is determined we must first consider the theory of concentration cells and electrode potentials. The fact that an electric potential difference is produced at the surface of contact of the metal with a solution is explained as follows: We know that the ions of metals are soluble because they are produced by the solution of metallic salts. There is also a store of electricity in the metal for charging the ions as illustrated by the behavior of radioactive metals. If a piece of metal is placed in pure water it gives off ions, but these ions taking positive charges from the metal leave the metal negative, and when the attraction of the negative metal for the positive ions equals the diffusion pressure (= osmotic pressure) of the ions no more ions will be given off. If instead of placing the metal in pure water we place it in a solution containing ions of the metal, the metal will give out a less number of ions and will become less negative. If the concentration of the metallic ions in the solution is increased, a point will be reached at which the metal will give out no ions and hence remain electrically neutral, and if the ionic concentration of the solution is still further increased ions will be deposited on the metal and give it a positive charge. If a piece of metal is placed in a solution containing 1 gram equivalent of its ions per liter it may become negative or positive according to the metal used. The following table is prepared in this way, but the neutral point (taken arbitrarily) is not the potential of the earth but the potential of a hydrogen electrode in a gram equivalent solution of hydrogen ions (see Abegg Auerbach & Luther, 1911). The potential that the electrode assumes is called the electrolytic solution tension.

Electrolytic Solution Tension.

Potential of metal (or non-metal) when immersed in a normal solution of its ions at 25°, as measured against a normal hydrogen electrode. Some American writers use reverse signs (+, —).

Cs		$\mathrm{Sn}^{\bullet\bullet}$	— .1	Pl	+ .789
$\mathrm{Li}^{\bullet}$	— 3.305	$\mathrm{Fe}^{\bullet\bullet}$	— .43	Pt	+ .863
$\mathrm{Rb}^{\bullet}$	— 3.205	$\mathrm{Tl}^{\bullet}$	— .32	$\mathrm{Au}^{\bullet}$	+ 1.5
$\mathrm{K}^{\bullet}$	— 3.203	$\mathrm{Co}^{\bullet\bullet}$	— .29	HSO_4'	+ 2.6
$\mathrm{Na}^{\bullet}$	— 2.993	$\mathrm{Ni}^{\bullet\bullet}$	— .22	SO_4''	+ 1.9
$\mathrm{Ba}^{\bullet\bullet}$	— 2.7	$\mathrm{Pb}^{\bullet\bullet}$	— .12	OH'	+ .41
$\mathrm{Sr}^{\bullet\bullet}$	— 2.767	$\mathrm{H}^{\bullet}$	0.00	Cl'	+ 1.35
$\mathrm{Ca}^{\bullet\bullet}$	— 2.55	As	+ .292	Br'	+ 1.08
$\mathrm{Mg}^{\bullet\bullet}$	— 1.55	$\mathrm{Cu}^{\bullet\bullet}$	+ .34	I'	+ .54
Al	— 1.276	Bi	+ .391	F'	+ 1.9
Mn	— 1.075	Sb	+ .466	S''	— .55
$\mathrm{Zn}^{\bullet\bullet}$	— .760	$\mathrm{Hg}^{\bullet\bullet}$	+ .86		
$\mathrm{Cd}^{\bullet\bullet}$	— .40	$\mathrm{Ag}^{\bullet}$	+ .8		

It may be seen from the table that the baser metals have a greater tendency to dissolve and produce ions than the noble metals.

Electrolytic solution tension is reduced by certain impurities in solution as follows, beginning with least effective: mannite <glycerine <glycol <meth. alc. <eth. alc. <acetone <prop. alc. <ether. (Abl, 1907.)

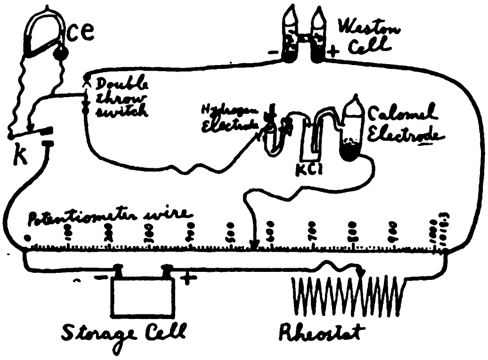


FIG. 7. Method of connecting the apparatus for determining hydrogen ion concentration (from MRR).

Determination of Hydrogen Ion Concentration with Hydrogen Electrodes

The apparatus, Fig. 7, consists of a hydrogen electrode, a vial, filled with a saturated solution of KCl, and electrolytically connected with the hydrogen electrode, a calomel electrode electrolytically connected with the vial by means of a syphon, a capillary electrometer, *ce*, with compound reading microscope,

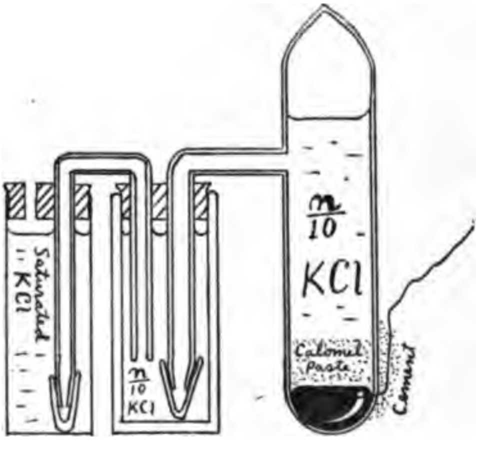


FIG. 8. Tenth normal calomel electrode (from MRR).

a double throw switch, a knife switch (not shown), a telegraph key, *k*, a potentiometer wire with two (or three) movable contacts, a Weston cell and a storage cell. The pieces of apparatus are connected by means of copper wires as shown in Fig. 7.

The 0.1 n calomel electrode, Fig. 8, is a test tube with syphon side neck, and a platinum wire or foil fused in the bottom. The platinum is plated or covered with pure mercury. Over the mercury is placed a layer of calomel (made by precipitation and washed repeatedly with a .1 n solution of KCl). The electrode is filled with the solution of KCl and the top closed so that the solution will not run out the syphon. The end of the syphon is closed with some porous material or an ungreased ground glass stopper or stopcock and immersed in a saturated solution of KCl, into which the stopcock of the hydrogen electrode also is dipped. An improvised electrode is shown in Fig. 9 (saturated type).

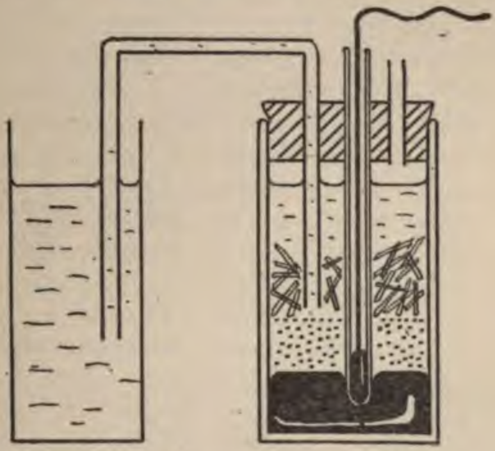


FIG. 9. Saturated calomel electrode (from MRR).

The capillary electrometer may be bought sealed or it may be made in the form shown in Fig. 7. Pure mercury is poured in so as to make contact with the fused in platinum wires in the wide and narrow arms, and about 30 per cent H_2SO_4 (by weight) is added until the capillary tube is filled. For accurate work this capillary must be fine as thermometer tubing and thin walled, so that higher power lenses may be used. The electrometer is clamped on the stage of a tilted back microscope. If much current is run through the electrometer, a drop of mercury must be allowed to pass from the capillary into the large arm.

The telegraph key short circuits the electrometer when not in use, and if a simple key is used instead, a short circuiting switch must be added.

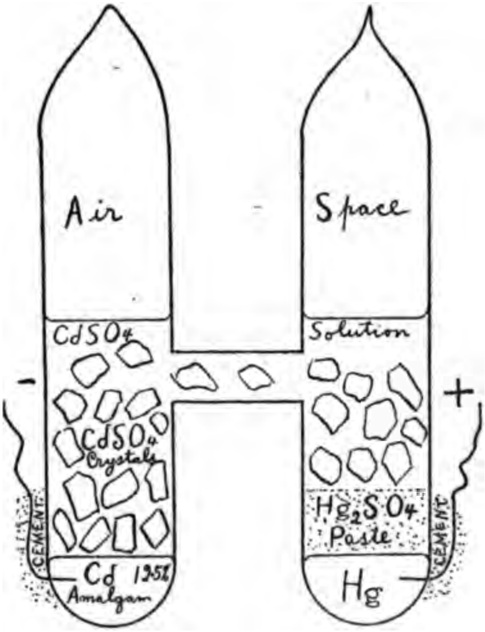


Fig. 10. Weston standard cell, saturated type (from MRR).

It is not usually economical to make a Weston cell (Fig. 10) if only one is needed and the materials are not already at hand. Directions for making it are given in Ostwald-Luther, "Messungen," 1910. Its voltage should be 1.0183 at 20°. If it is short circuited it will not recover for several days.

The potentiometer should have a high electrical resistance in order to prevent the storage cell from running down rapidly, and to reduce errors due to contact resistances within itself.

If a standard potentiometer or one like Fig. 11 is not available a No. 36 nichrome wire stretched over a 1018.3 millimeter scale may be used, but it must be calibrated as the meter wire is in Ostwald-Luther, "Messungen," 1910.

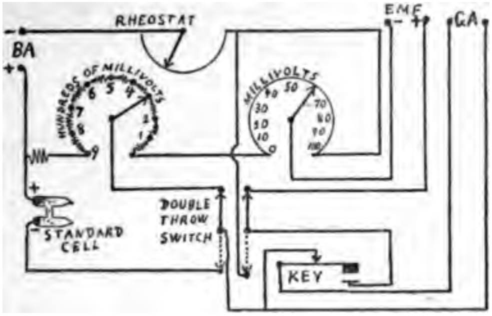


Fig. 11. Potentiometer and standard cell combined (L and N) from MRR.

The principle of the potentiometer is as follows: Suppose we have a wire stretched over a scale divided into equal divisions, and an electric current flowing through the wire so that each division has a fall of potential of one millivolt. If we lead off a shunt circuit from two points on the wire 10 divisions apart through an electrometer, a current of 10 millivolts will flow through the electrometer. Interpose in the shunt circuit an emf of 10 millivolts in a direction opposite to the current in the shunt, no current will flow through the shunt, and there will be no deflection in the electrometer. Interpose an unknown emf in the shunt and move the contacts on the potentiometer wire until no deflection is seen in the electrometer, and the voltage of the unknown is equal to the number of divisions between the contacts of the shunt leads, in millivolts. In order to standardize our current, we reverse this process by placing a known emf (Weston cell) in the shunt and then changing the total length of the wire until each division equals a millivolt.

The hydrogen electrode is essentially a metal electrode saturated with hydrogen. Platinum absorbs hydrogen greedily especially when in the finely divided state known as platinum black, and is excellent for the surface of the electrode. But it is better to make the interior of gold so that it will not absorb so much hydrogen and take it away from the surface. The gold is covered with platinum black as in platinizing conductivity electrodes, but it is not necessary to remove Cl_2 by H_2SO_4 and electrolysis, as the electrode is never used as anode and no Cl_2 is formed. In order to keep the electrode saturated with hydrogen while it is immersed in the fluid to be investigated, special vessels are necessary, but these are usually inseparably connected with the electrode and included under the term.

Blood requires more precautions than any other biological fluid when investigated with the hydrogen electrode. When success has been achieved with it, the same technique and apparatus will serve for any other biological fluid with slight omissions and modifications for expediency. Though many forms of electrodes have been described, but four points in the technique need be emphasized: First, reduce the layer of blood over the platinum as much as possible so that hydrogen can get to the platinum (Michaelis "Wasserstoffionenkonzentration," 1914). Second, shake the hydrogen with another sample of the same blood before passing it into the electrode, otherwise CO_2 will pass from the blood into the hydrogen and leave the blood more alkaline (Hasselbalch, 1913). Third (not absolutely necessary), remove the erythrocytes. Otherwise the oxygen they contain will combine with the hydrogen in the platinum and reduce its concentration (Milroy, 1914). Fourth, connect the apparatus so that the blood is never exposed to the air and CO_2 lost (McClendon 1916 a).

FIG. 12. Hollow needle for drawing blood (from JBC).

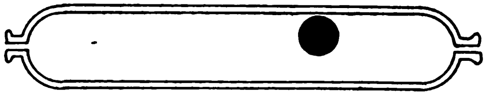


FIG. 13. Tube for collecting, defibrinating and centrifuging blood without exposure to air. The ends are fitted with rubber tubes (from MRR).

The following apparatus is the first to include all of these points (McClendon & Magoon, 1916).

The blood is drawn through a hollow needle, Fig. 12, connected by rubber tubing to the 10 cc defibrination tube, Fig. 13, and defibrinated with the lead ball with the exclusion of air. This tube is then placed in the centrifuge and the corpuscles precipitated. The lower end is connected to a mercury tube and funnel, with the exclusion of air, and the upper end is connected to the Fresenius cock *a* of the electrode, Fig. 14, that has already been rinsed with distilled water and filled with pure hydrogen. Rais-

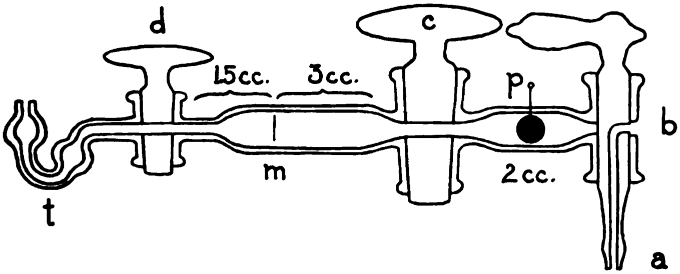


FIG. 14. Hydrogen electrode for fluids containing CO_2. Hydrogen is shaken with one portion of the fluid in the large compartment and passed into another portion of the fluid in the small compartment without exposure to air (from JBC).

ing the mercury funnel causes the serum to rise in the cock and wash out the air, and when this is done the cock is turned (the two others likewise) so that the serum enters the electrode, the hydrogen bubbling out through the water in the trap, *t*. The 2 cc chamber is completely filled with serum, the 4.5 cc chamber filled until it contains only 1.5 cc of hydrogen and all of the cocks closed. The electrode is shaken or inverted 200 times, to bring the hydrogen to equilibrium as regards CO_2. The middle cock is now opened and the electrode held vertically or shaken so that the hydrogen displaces 1.5 cc serum from the smaller chamber. In passing from the large to the small chamber, the hydrogen is in contact with the returning serum spread in a thin film, and so completes CO_2 equilibrium. The middle cock is closed and the electrode shaken or inverted 200 times, connected to the potentiometer by means of a wire hooked into the platinum loop, and to the calomel electrode by dipping the Fresenius cock into the vial filled with a saturated solution of KCl. Clark and Lubs (1916) state that it is better to have the KCl meet the serum in a wide tube and make this contact immediately before taking the reading. I have not found this precaution necessary for an accuracy of 1 millivolt. If desired, the precaution may be taken by having the opening, *a*, Fig. 14, wide, lubricating this stopcock with serum before filling the electrode and leaving the outlet filled with serum after filling the electrode. The temperature of the room is controlled or at least noted. A convenient thermoregulator for the room is shown in Fig. 15. The first

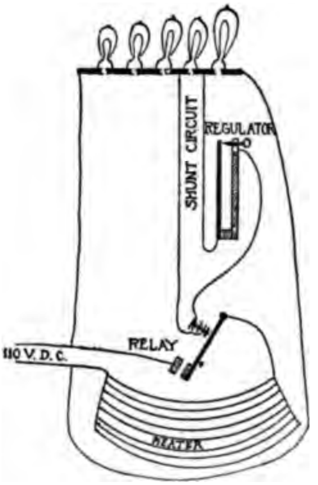


Fig. 15. Thermoregulator for maintaining constant temperature in a room. The regulator is of strips of invar and brass welded together, and the heater is of nichrome wire strung through the air. An electric fan is necessary (from JBC).

reading on the potentiometer is correct provided the electrode is the temperature of the room. If more readings are taken the electrode should be shaken or turned so as to splash a fresh layer of serum over the platinum. The efficiency of this electrode is shown by the fact that successive readings during one hour (or even forty-eight hours) are the same within 1 millivolt. The electrode is cleaned with potassium bichromate in sulphuric acid and left filled with distilled water. Before use, it is replatinized a few seconds, rinsed thoroughly with distilled water, the Fresinius cock lubricated with serum or KCl solution and the electrode filled with pure hydrogen. Venous blood may be used in the electrode instead of serum, provided hirudin or oxalate are used to prevent coagulation.

If it is desired to measure the PH of the serum at a known CO_2 pressure, the electrode is sealed to a Hemphill burette. Since CO_2 pressures above 10 per cent of an atmosphere are not desirable, the 100 cc burette may be shortened, and only 10 cc made narrow and graduated as in Fig. 16. This burette may be called a tonometer, since the pressure of CO_2 in atmospheres is measured

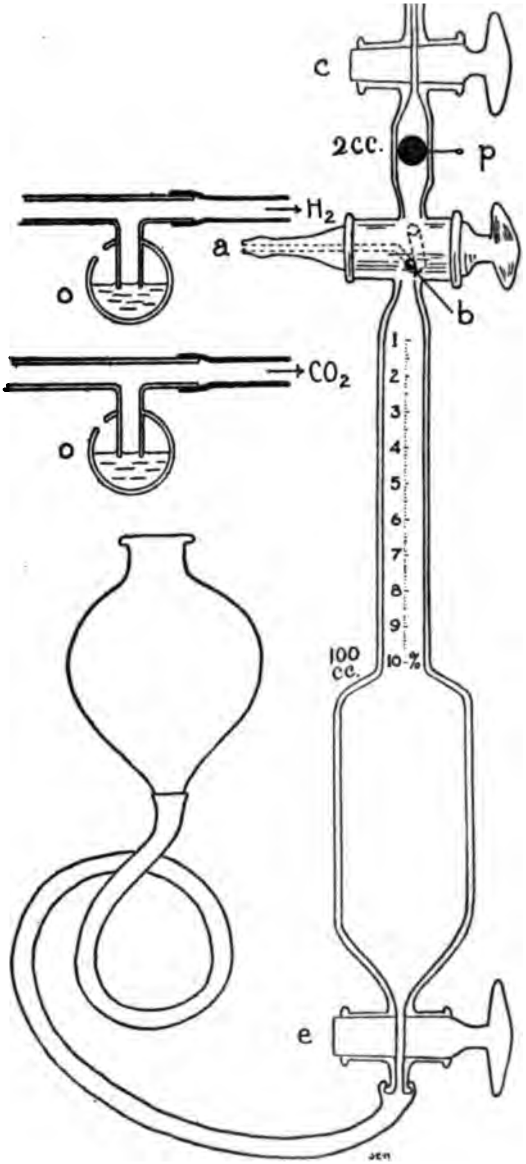


Fig. 16. Combined tonometer and hydrogen electrode. The fluid is passed from the 2 cc electrode into the 100 cc tonometer and back into the 2 cc electrode (from JBC).

by it. The electrode is first filled with serum through *a*. The apparatus is set up as in the figure, and the tonometer filled with mercury by raising the mercury funnel. The connections with the H_2 and CO_2 generators are provided with side tubes with traps, *o*, to make sure that the gas issuing from them never varies more than about a millimeter of water from atmospheric pressure. The three way cock, *a*, is connected with the CO_2 generator, its outlet washed through the opening, *b*, and the cock turned so as to admit CO_2 into the tonometer while the funnel is being carefully lowered. When the right per cent of CO_2 is read off on the graduations the lower cock, *e*, and three way cock, *a*, are closed. Hydrogen is admitted in the same way as was the CO_2, in order to fill the tonometer with gas at atmospheric pressure, and the lower cock, *e*, closed. If it is desired to make gas mixtures containing less than 1 per cent CO_2, for example 0.03 per cent CO_2, first make 1 per cent CO_2, mix by allowing to stand or inverting the apparatus, and expel all but 3 cc by raising mercury funnel, then fill with hydrogen. The apparatus is removed, the three way cock, *a*, is so turned as to connect the tonometer with the electrode, the serum shaken down into the tonometer and rotated fifteen minutes. One half cc of serum is shaken back into the electrode, connections made and reading taken. If it is desired to use another CO_2 pressure on the same serum, all of the serum is shaken into the electrode and the process repeated. By measuring the PH of the serum at CO_2 tension of the alveolar air, the PH of the arterial blood may be obtained. It is not always possible to distinguish between the PH of venous and of arterial blood, because the slight difference may come within the limit of error of the method, but since the PH of arterial blood is more constant than that of venous blood, the significance of this procedure is obvious.

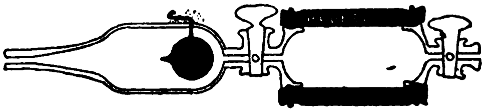


FIG. 17. Hydrogen electrode for CO_2-free fluids (from MRR).

A simple hydrogen electrode for small quantities of fluid is shown in Fig. 17. If used for titrations the rubber bulb may

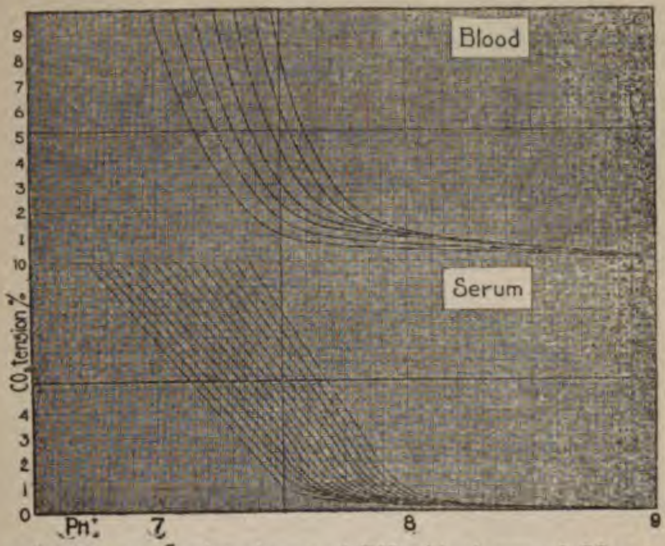


FIG. 18. Curves showing change of PH with change of CO_2 pressure. The CO_2 tension is in percentage of atmosphere (from JBC).

be replaced by a side outlet with stopcock and the fluid forced into the electrode by immersion in the beaker.

By the buffer value or reserve alkalinity of the blood, we mean its resistance to change of reaction. Since the buffer value is always expressed in arbitrary units, varying with the method of determination, those values only are comparable that are obtained by the same method. The buffer value of serum, although not the same as that of blood, is approximately proportional to it, and hence may be used in place of it (see Fig. 18). Since CO_2 is the principal reagent used by the organism in maintaining the reaction of the blood, it is of interest to know the change in reaction of the blood with change in CO_2 pressure. This is shown in the curve in Fig. 18. It may be noticed that within the normal physiological limits of CO_2 tension the change in reaction is very small (the normal curve passing through $PH = 7.5$ and $CO_2 = 5.2$ per cent). It is therefore necessary to take two very different CO_2 pressures in order to obtain a reliable buffer value. But since the PH of blood in the body is rarely far from 7.5, the whole curve may be read from Fig. 15 provided merely the CO_2

pressure of blood (or alveolar air) is known or the PH of blood at any known CO_2 pressure is determined.

It should be noted that hydrogen electrodes are "poisoned" by H_2S, NH_3, Cl_2 and arsene, therefore the hydrogen should be freed from arsene by passing it through potassium permanganate+KOH solution and through mercuric chloride solution. If H_2 is produced electrolytically the O_2 should be removed (Clark and Lubs, 1916). Putrescent solutions are best studied by Rowntree's method (see indicators).

Since the potentiometer measures the emf in millivolts, this reading must be converted into the PH by the following formula:

$$PH = \frac{\text{emf} - 337}{.1984\ T}, \text{ where } T = \text{absolute temperature.}$$

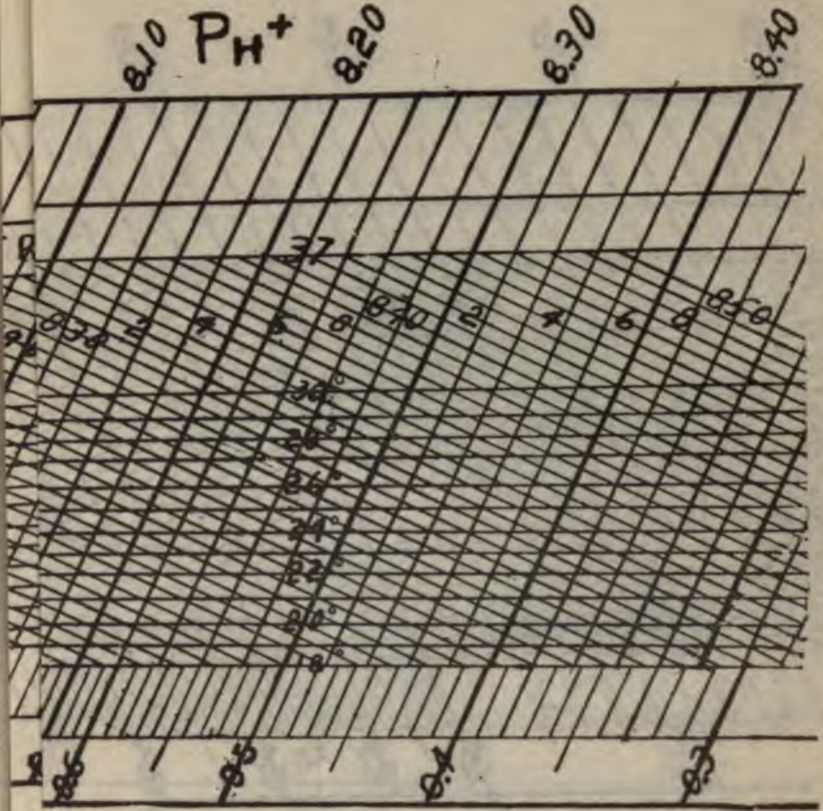
Or the conversion table, Fig. 19, may be used.

The following data will assist in constructing a conversion table like Fig. 19 of wider range:

For PH = 0	For Ph = 10			
Emf = 337 from 18° to 32°	Emf	Temp. degrees	Emf	t°
336.9 at 33°	914.4	18	932.3	27
336.8 34°	916.4	19	934.3	28
336.7 35°	918.4	20	936.2	29
336.6 36°	920.4	21	938.2	30
336.5 37°	922.4	22	940.2	31
	924.3	23	942.2	32
	926.3	24	944.1	33
	928.3	25	946.	34
	930.3	26	947.9	35
			949.7	36
			951.6	37

For conversion tables for the saturated calomel electrode see McClendon (1916 b).

The formula and conversion table refer to the emf obtained with the partial pressure of hydrogen in the electrode = 760 mm, but since this is lowered by N_2, CO_2, water vapor, and by the barometric deficiency (760 — bar.) the potentiometer reading must be increased by $(30 \log \frac{760}{q})$ where q = partial pressure of hydrogen in the electrode. According to Clark and Lubs (1916) a correction should also be applied for the expansion of H_2 by heat. This would make the number 338 apply to a wide


8.10
PH+
8.20
8.30
8.40
37
8.30
2
4
6
8
8.40
2
4
6
8
8.50
30°
28°
26°
24°
22°
20°
18°
8.6
8.5
8.4
8.3

range of temperatures. All these corrections are combined in the following table from Clark and Lubs:

Correction in millivolts to be added to potentiometer reading, for change in concentration of hydrogen due to barometric pressure, temperature and vapor tension of water.

Barometer (mm)	20°	25°	30°
780	0.85	1.19	1.57
770	1.02	1.36	1.74
760	1.19	1.53	1.92
750	1.36	1.71	2.10
740	1.53	1.89	2.28

Determination of Hydrogen Ion Concentration by Means of Indicators

According to Ostwald, indicators are weak acids or bases whose ions are not colored like the undissociated molecules. Indicators may, however, be used in titration in non-aqueous solvents in which dissociation is very slight. The theory of Stieglitz that the change in color is due to intramolecular change, following change in ionization, may account for the fact that the change in color may not be instantaneous. It is possible that the amount of hydration of the ions has an effect on their color, since the presence of neutral salts affects the color of indicator solutions.

The following indicators and PH at which they change are given by Friedenthal:

Indicator	PH
Alizarin	6- 8, 11-15
Alizarin blue S	11-13, 7- 8
Alizarin green B	12-13
Alizarin Na sulfonate	4- 6
Alkali green	3- 7
Azolilmin (litmus)	5- 9
Benzopurpurin B	0- 5, 13-14
Bitteralmondoilgreen	0- 2, 11-14
Cochineal	4- 6
Congo red	3- 5
Crocein	12-14
Curcumein (Tumeric)	13-15
Cyanin	6- 8
Dimethylamidoazobenzol	2- 4
Echtrot	0- 1
Eosinmethyleneblue	0- 3, 14-15
Fluorescein	0- 6
Gallein	1- 6, 10-14
Guaiac	7- 8
Haematein	0-15
Heliantin I	11-13
Heliantin II	12-14
Iodeosin	0-15

Indicator	PH
Lacmoid	4- 8
Magdalarot 3-4, 14-15	3- 4, 14-15
Mauvein	0- 3, 13-15
Methyl green	0- 2, 11-14
Methyl orange	2- 5, 14-15
Methyl violet	0- 3, 12-15
a-Napthol benzoin	9-11
Neutral red	0- 1, 7- 9
p-Nitrophenol	5- 7
Phenacetolin	3- 6, 10-13
Phenolphthalein	8-10, 13-15
Rosolic acid	6- 8, 13-15
Safranin	0- 1, 14-15
Saurefuchsin (Acid fuchsin)	9-14
Tetrabromphenolphthalein	8- 9, 12-15
Thymolphthalein	10-11
Tropaeolin	7- 9
Tropaeolin o	11-13
Tropaeolin oo	0- 3
Tropaeolin ooo	11-13
Trinitro benzol	12-15

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The following indicators were found by Sörensen (1909) to be least affected by proteins, but some control is necessary if the protein content of the unknown solution is considerable.

Indicator	PH
Methyl violet	0.1— 3.2
Mauvein	0.1— 2.9
(Methyl red) o—Benzolcarbonicacid azo dimethyl anilin	4.2— 6.3
p—Nitro phenol	5 — 7
Neutral red	6.8— 8
Rosolic acid	6.9— 8
(Tropaeolin ooo) p—Benzolsulfonicacidazo-a-napthol	7.6— 8.9
a—Napthol phthalein	7.3— 8.7
Phenolphthalein	8.3—10
Thymolphthalein	9.3—10.5

The following indicators were studied by Clark and Lubs (1915):

Indicator	PH
Monomethyl red	4.25—6
Monoethyl red	4.25—6
Diethyl red	4.5 —6.5
Monopropyl red	4.25—6.25
Dipropyl red	4.5 —6.5
Dimethyl-a-napthylamine red	5. —6.75
Phenolsulphonephthalein	6.5 —8.5
o—Cresolsulphonephthalein	6.5 —8.5
Thymolsulphonephthalein	8. —9.75
a—Naptholsulphonephthalein	7.5 —9.
Tetrabromphenolsulphonephthalein	3.5 —4.5
Dibromthymolsulphonephthalein	6. —7.25

The color change and PH of solutions of some useful indicators were shown in a colored chart. In order to find the PH of an unknown solution, pour it into a test tube of 1 cm bore, add 0.1 cc of the indicator solution, and while looking down into it against a white background, compare it with the chart. If the test tube is 2 cm bore, .4 cc of indicator solution should be used. (McClendon, 1916, b; also in Tortugas Vol., Carnegie Ins. Wash., in press.)

As shown by Sörensen (1909) some indicators are more sensitive than others to neutral salts, proteins and protein decomposition products. Congo red and litmus are therefore to be especially shunned in biological work. For solutions containing large amounts of proteins, or having a color of their own, the method of Rowntree, Marriott and Levy, 1915, is useful, provided that obvious extra precautions are recognized. The fluid to be tested is placed in a dialyzing capsule of collodion or parchment paper impermeable to protein, and introduced into a test tube containing some neutral (boiled) distilled water or salt solution. The test tube is stoppered to prevent the loss of CO_2 and dialysis allowed to proceed to approximate equilibrium. The capsule is removed and the right quantity of indicator added. The color is compared with that of a series of tubes of the same bore, containing the same amount of indicator and solution but of known PH. (Such tubes may be obtained, sealed, from Hynson, Westcott and Co., Baltimore.) Or the tube may be compared with the colored chart. One effect of dialysis is to dilute the solutes affecting the PH and causing an error which is less the greater the buffer value of the solution.

Buffers and Solutions of Standard Hydrogen Ion Concentration

The PH of water, or solutions of neutral salts, or very dilute solutions of strong acids or alkalis, is changed by minute traces of certain impurities. Absolutely pure water (conductivity water) is neutral but may not remain so. If exposed to the air it becomes acid due to absorption of CO_2, and if kept in glass it becomes alkaline, due to solution of the glass. "Pyrex" or "nonsol" glass is better in this respect, but any glass is improved by allowing a jet of steam to condense on the surface and drain off, a process known as steaming out. Silica or metal vessels are more reliable. This difficulty in maintaining fixed hydrogen ion concentrations is obviated by the use of buffers. The chief buffer in organisms is $NaHCO_3$. The reaction of a pure solution of $NaHCO_3$ is but very slightly alkaline, that of Na_2CO_3 somewhat more alkaline and that of CO_2 but slightly acid. If we add a strong acid to a solution of $NaHCO_3$ the acidity of the mixture will not exceed that of a solution of CO_2 until all of the $NaHCO_3$ has been decomposed. If we add a strong alkali to a solution of $NaHCO_3$ the alkalinity of the solution will not exceed that of a Na_2CO_3 solution until all of the $NaHCO_3$ has been decomposed. One drop therefore of n HCl would acidify a liter of H_2O or neutral salt solution more than a liter of the acid would acidify a liter of m $NaHCO_3$ solution—hence the advantage of a relatively high concentration of buffers in standard solutions. The standard solutions used by Sörensen (1912) are shown in the chart in Fig. 20. On the ordinate is given the number of cc of the first solution of a pair to be taken, when the second solution is to be added to give a total volume of 10 cc. The stock solutions are as follows:

"HCl" = A 0.1 n solution of HCl titrated with silver nitrate.

"NaOH" = A 0.1 n solution of NaOH made by adding an excess of sodium or sodium amalgam to CO_2-free H_2O and titrating with HCl and diluting to .1 n. Or decanting a clear saturated solution of NaOH and diluting to required volume with CO_2-free H_2O.

"Citrate" = A 0.1 m solution of secondary sodium citrate made by dissolving 21.008 g citric acid in 800 cc of .25 n NaOH and diluting to a liter. A layer of ether on top will preserve it.

"H_3PO_4" = A 1/15 m solution of H_3PO_4 accurately standardized.

"KH_2PO_4" = A 1/15 m solution of KH_2PO_4 made by dissolving 9.078 g of the recrystallized anhydrous (desiccated) salt to the liter.

"Na_2HPO_4" = A m/15 solution of Na_2HPO_4 made by dissolving 11.876 g to the liter, of $Na_2HPO_4 2H_2O$ that has been recrystallized and dried until it has the right water content by analysis.

"Borate" = A solution of 12.404 g of recrystallized desiccated boric acid + 100 cc n NaOH solution made up to 1 liter.

The distilled water used in making these solutions should be free from NH_3 and CO_2. The CO_2 may be practically eliminated by boiling 15 minutes in a tin vessel and cooling under a soda lime tube. All solutions should be kept in "nonsol" or "pyrex" glass vessels or in paraffined bottles and those with PH above 5 should be provided with soda lime tubes. The reagents should be the purest. In dropping metallic sodium into water O_2 should be excluded. NaOH made from Na may be purchased but should be freed from carbonate by making it first an 80 per cent solution in a stoppered bottle and allowing the carbonate to precipitate, then pipetting off the clear solution, diluting with CO_2-free water and standardizing it. All of the salts should be dissolved in boiling H_2O, filtered and crystallized at least once. It is difficult to obtain pure phosphates. The Na_2HPO_4 solution should turn phenolphthalein a deep red. In the chart, Fig. 20, impurities in the reagents affect the ends of the curves more than the middle.

The buffer mixtures of Clark and Lubs (1916) are as follows:

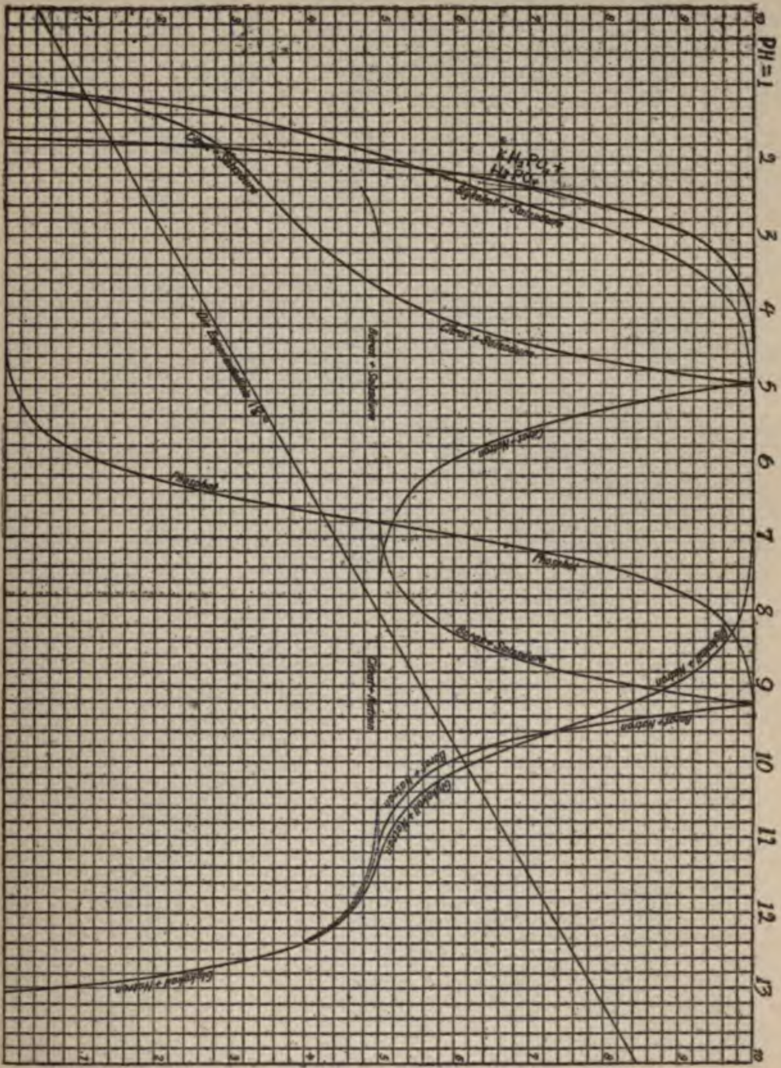


FIG. 20. Sörensen's chart of buffer mixtures. On the ordinate is the number of cc of the first solution when the mixture is completed to 10 cc with the second solution indicated on the curve. On the abscissa is the PH at 18° (from EP).

1. Solutions made up to 200 cc and containing 50 cc 0.2 m KH-phthalate +

0.2 n HCl	46.70	39.60	32.95	26.42	20.32	14.70	9.90	5.97	2.63
PH at 20°	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8

2. Solutions made up to 200 cc and containing 50 cc 0.2 m KH-phthalate +

0.2 n NaOH	0.40	3.70	7.50	12.15	17.70	23.85	29.95	35.45	39.85	43.00	45.45	47.0
PH at 20°	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2

3. Solutions made up to 200 cc and containing 50 cc 0.2 m KH_2PO_4 + 50 cc 0.2 n KCl +

PH at 20°	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
0.2 n NaOH	3.72	5.70	8.60	12.60	17.80	23.65	29.63	35.00	39.50	42.80	45.20	46.80

4. Solutions made up to 200 cc and containing 50 cc of 0.2 m H_3BO_3 +

0.2 n NaOH	2.61	3.97	5.90	8.50	12.00	16.30	21.30	26.70	32.00	36.85	40.80	43.9
PH at 20°	7.8	8.0	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0

These solutions should be made from the purest reagents, that have been recrystallized three times. The acid potassium phthalate, acid potassium phosphate, and potassium chloride may be dried at 110° but the boric acid must be dried at room temperature in a desiccator.

The more acid of the mixtures will deposit crystals of phthalic acid if not kept considerably above 20°.

It may be inferred that the buffer value of these solutions is less than those of Sörensen, but their salt action on indicators is also less.

Dissociation Constants of Acids and Bases

Acids and bases differ from neutral salts in that among their dissociation products are the ions of water, $H^{\cdot}$ or OH' as the case may be. Since the strength of acids and bases lies in the number of H and OH ions they dissociate, it is determined by the dissociation constant, c.

From the law of mass action we have: $c = \frac{a^2}{v(1-a)}$, where v is the number of liters in which 1 mol of the acid (for instance) is dissolved, $1-a$ represents the proportion of the undissociated molecules and a represents the proportion of anions = cations. If we increase the dilution, v, then a^2 increases and therefore ($a = H^{\cdot}$) increases. ($H^{\cdot}$ per liter decreases.)

Strong acids appear to disobey this law when dissociation is determined by electric conductivity. If c is determined from the dissociation of a more concentrated solution, a more dilute solution is found to be dissociated less than is calculated from the formula. In other words, the dissociation in concentrated solutions is more than is expected. The anomaly may be explained by the hypothesis that the hydration of ions is less in the concentrated solution, they move faster and increase the conductivity, and, hence, the high dissociation is only apparent (G. N. Lewis). The dissociation of concentrated solutions of HCl as calculated from electrode potential (J. Ellis, 1916) is much greater than that calculated from conductivity data. The two curves for dilute solutions are shown in Fig. 21.

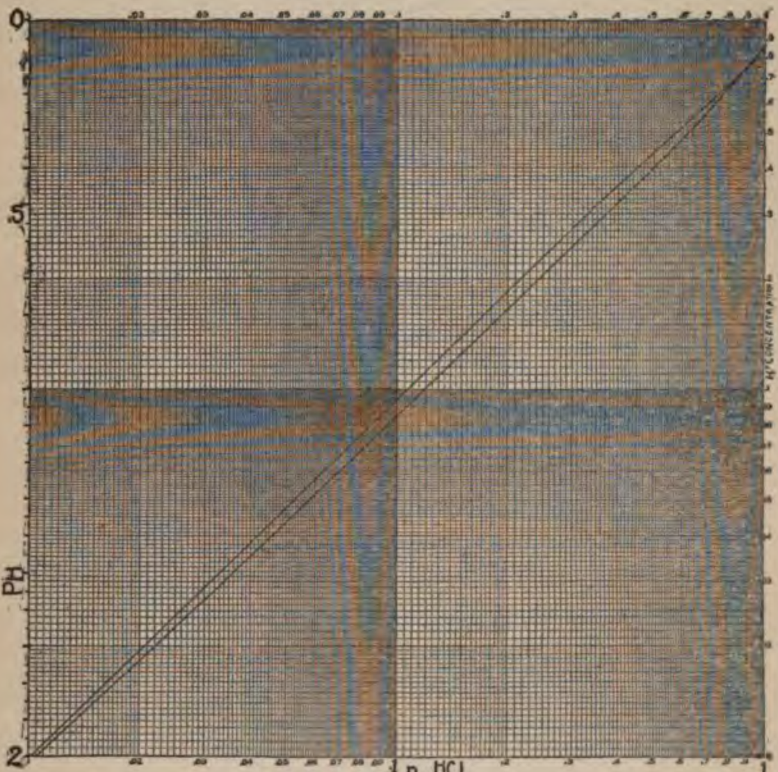


FIG. 21. Curves showing the difference between the dissociation of HCl as measured by electric conductivity (conductance ratio) and electrode potential (hydrogen ion concentration).

In a molecular solution of an acid $v = 1$ and the formula becomes: $c = \frac{a^2}{a-1} = \frac{H^{\bullet} \times A'}{HA}$, where $H^{\bullet}$ and A' represent the concentration of the ions and HA of the undissociated molecules of the acid.

The dissociation constants of some weak acids and substances which dissociate traces of $H^{\bullet}$, are as follows at 25° (mostly from Scudder, 1915):

—log K_A	Acid	—log K_A	Acid
— .92	Trichloracetic	10.22	Carbonic (CO_3'')
+1.00	Oxalic	11.00	Chloral hydrate
1.29	Dichloracetic	11.74	Maltose
1.96	Phosphoric (H_2PO_4')	12.06	Levulose
2.81	Monochloracetic	12.23	Dextrose
2.96	Salicylic	12.23	Lactose
3.01	Tartaric	12.43	Arabinose
3.08	Citric	12.44	Phosphoric (PO_4''')
3.40	Malic	12.72	Sucrose
3.65	Hippuric	13.47	Mannite
3.68	Formic	14.15	Glycerol
3.82	Acetoacetic	14.74	Propionitrile
3.85	Lactic		
4.17	Succinic	—log K_B	Base
4.18	Benzoic	2.80	Piperidine
4.25	Acrylic	2.90	Diethylamine
4.44	Adipic	3.25	Ethylamine
4.49	Gallic	3.27	Dimethylamine
4.7	Betaoxybutyric	3.39	Methylamine
4.73	Acetic	4.07	Ethylenediamine
4.80	Butyric	4.22	Trimethylamine
4.82	Valerianic	4.34	Allylamine
4.84	Propionic	4.74	Ammonia
4.84	Caproic	4.77	Brucine
4.84	Caprylic	5.58	Quinine
5.80	Uric	6.07	Strychnine
5.80	Salicyl aldehyde	7.00	Atropine
6.00	Tannic	7.00	Pilocarpine
6.05	Phosphoric (HPO_4'')	8.64	Pyridine
6.19	Cacodylic	9.30	Aniline
6.52	Carbonic (HCO_3')	9.50	o-Toluidine
7.24	Hydrogen sulphide	9.58	Methylaniline
9.24	Boric (H_2BO_3')	9.62	Dimethylaniline
9.33	Hydrocyanic	9.80	Ethylaniline
9.92	Phenol	13.80	Urea

The temperature coefficient for the dissociation of acids and bases is usually small.

In case of CO_2 and NH_3, the above figures show only the apparent dissociation constants. The real acid and base are H_2CO_3 and NH_4OH, but the concentration of these is unknown. According to the investigations of Thiel and Strohecker (1914) the dissociation constant of H_2CO_3 is greater than that of formic acid.

Dissociation of Amphoteric Electrolytes (Ampholytes)

Ampholytes dissociate both H and OH ions, having therefore two dissociation constants, K_A and K_B. A pure solution of an ampholyte has an acid reaction, if K_A is the greater, and an alkaline reaction if K_B is the greater. The following table gives the dissociation of some amino acids and dipeptides at 25°. According to these figures, mostly from Scudder (1914), the dipeptides are dissociated to a greater extent than the amino acids from which they are formed, and the dissociation of $H^{\cdot}$ has increased more than the dissociation of OH', making the dipeptides more strongly acid than the amino acids.

$-\log K_A$	Ampholyte	$-\log K_B$	PH of Isoelectric Point
3.85	Aspartic acid	11.92	3.03
4.39	Glutamic acid	.....	.....
4.79	m-Aminobenzoic acid	10.91	3.79
4.92	p-Aminobenzoic acid	11.63	4.20
7.74	Glycylglycine	10.70	5.52
7.74	Alanylglycine	10.70	5.52
7.82	Leucylglycine	10.52	5.66
8.16	Taurine	.....	.....
8.28	Asparigine	11.74	5.27
8.40	Tyrosine	11.58	5.41
8.66	Phenylalanine	11.89	5.35
8.66	Histidine	8.24	7.21
9.70	Alanine	11.28	6.72
9.74	Glycine	11.55	6.58
9.74	Leucine	11.49	6.54
11.00	Lysine	7.00	9.00
13.96	Arginine	7.00	10.48

These dissociation constants show the relative dissociation of H and OH ions by an ampholyte when dissolved in a neutral solute. But the addition of an acid to the solution will reduce the dissociation of H ions and the addition of a base will reduce its dissociation of OH ions by the ampholyte, according to the law of mass action. At a certain reaction of the solution for each ampholyte, the dissociation of H and OH ions will be equal. The "remainder" of the molecule will be therefore neither electronegative nor positive, but neutral or isoelectric. The reaction at which this occurs is called the isoelectric point. According to Michaelis the PH of the isoelectric point is one half of $(-\log K_W)+(-\log K_A)-(-\log K_B)$ and the PH so calculated agrees with the PH found by experiment. Isoelectric points are given in above table, those of proteins in Chapter VI.

CHAPTER V

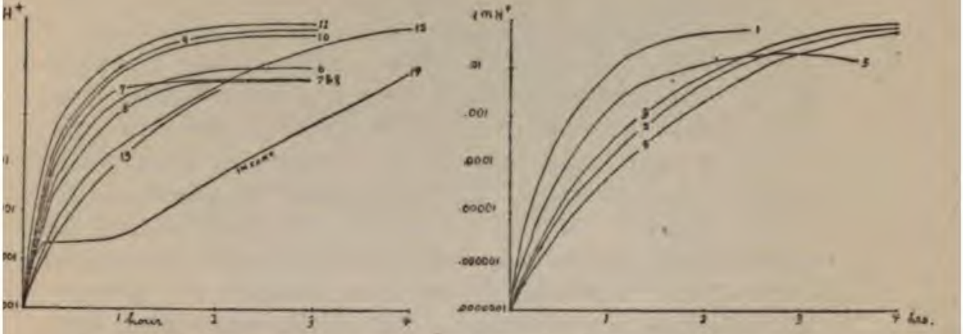
SURFACE TENSION AND ADSORPTION

We are sometimes surprised at the strength of the surface film when we see insects supported on the surface of water, but according to Colton (1910) a small clam suspended itself by threads (byssus) attached at only three points from the surface film in the Naples aquarium. This is more surprising when we realize that the thickness of the film is .00000006 mm.

Although instances were already known, it was first formulated by J. Willard Gibbs (1874) into a general rule that substances which lower the surface tension of a solvent become more concentrated in the surface film than in the interior. Take for example a system of two phases, oil and water. If a substance which lowers the surface tension of water be dissolved in the water it will concentrate in the surface film, next to the oil. If the substance be soluble in oil, it will diffuse into the oil until an equilibrium is reached between the concentration of the substance in the oil and in the surface film of the water. If this substance therefore has the same solubility in water and in oil and does not lower the surface tension of oil it will become more concentrated in the oil than in the water. If the substance be insoluble in the oil it will remain concentrated in the film of water next to the oil and is said to be adsorbed by the oil. Hence, surface tension, adsorption and diffusion are related phenomena.

In the phase boundary between two fluids it is easier to measure the surface tension than the adsorption. On the contrary it is impossible to measure the surface tension in the phase boundary between a fluid and a solid, but the adsorption may be measured, actually or relatively.

The most practicable method of measuring surface tension, in biochemistry, is the estimation of the average weight of a drop of the one of the fluids falling from a standardized pipette into the other fluid. In most measurements one of the fluids



Charts showing the hydrogen ion concentration of the stomach (on the ordinate) and time after eating (on the abscissa). Curves 1-4 from same man with different quantities of food. Curve 14 is the average curve for infants.

is air, and the drop is hence allowed to drop out of the pipette into the air. The diameter of the dropping surface = 4.6 to 5.7 mm (J. L. Morgan, 1915). One form of pipette is Traube's (1904) stalagmometer. In its use, the weight of the drop is found by multiplying the volume of the drop by its specific gravity, the volume of the drop found by dividing the volume of the pipette by the number of drops from one filling. The pipette is standardized with water, whose surface tension is taken as unity. Therefore the surface tension =

$$\frac{\text{specific gravity of solution} \times \text{no. of drops of water}}{\text{number of drops of solution}}$$

Traube's (1912) viscostagmeter is a dropping burette with which the volume of say ten drops is measured, and is more rapid than the stalagmometer, which holds forty to 100 drops of water. (Stalagmometers are made to order by Kimball Durand Co., Chicago.)

It is the presence of the substance in the surface film which lowers the surface tension, but it is not until diffusion into the surface film has reached an equilibrium that the lowest surface tension is present. For instance, soap very greatly lowers the surface tension of water, but when measurements are taken on new surfaces by the instantaneous method of Rayleigh, the surface tension of the soap solution is found to be the same as that of water. If the soap solution is allowed to drop slowly from a stalagmometer it shows a lower surface tension than if dropped fast. The rate of flow from a stalagmometer is reduced by a piece of capillary tubing forming part of the outflow tube, but the more viscous the fluid the slower it will flow. In order to reduce errors from this source, Traube uses three stalagmometers for solutions of different viscosity and selects one delivering less than one drop per second. The error in using the stalagmometer, due to lack of diffusion equilibrium, is less than the error in the methods used on old surfaces, due to the formation of haptogen membranes. It should be remembered, however, that the stalagmometric data are merely comparative, and only those results should be compared where the flow is slow and its rate approximately the same in each case (see Harkins and Humphrey, 1916).

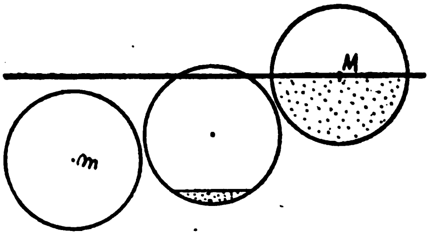


FIG. 22. Scheme showing that surface tension is located in a film whose thickness equals the diameter of the sphere of molecular attraction.

In order to understand the relation between surface tension and osmotic pressure certain theoretical considerations are necessary. Surface tension is a molecular phenomenon. The thickness of the surface film equals the radius of the sphere of molecular attraction. In Fig. 22, suppose *m* to represent a molecule in the interior, *M* a molecule on the surface of a liquid, and the circles around them to define the spheres of molecular attraction. The sphere of *m* is entirely within the liquid, hence *m* attracts and is attracted equally on all sides by all molecules in this sphere. On the contrary, only the hemisphere of *M* is within the liquid and, hence, *M* is attracted laterally and downward, but not upward. This attraction which the molecules in the surface film have for one another puts the film under a tension—surface tension.

If the surface be convex the surface tension is greater, increasing with the convexity. In Fig. 23, *m* and *M* are two molecules equidistant beneath a plane surface (*m*) and a convex surface (*M*). These molecules attract and are attracted by the molecules within the lightly shaded areas, the spheres of molecular attraction. The uncompensated downward attractions are represented by the more deeply shaded areas. The area is larger in the convex film and, hence, the surface tension greater.

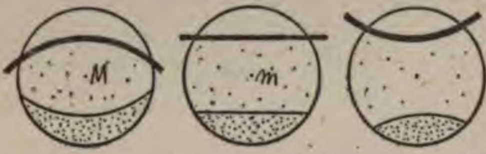


Fig. 23. Scheme showing that convexity increases surface tension and concavity decreases it.

The interior of a drop of a liquid is under a hydrostatic pressure due to its surface tension, the pressure being greater the smaller the drop. Imagine a spherical drop bisected by a plane. If the surface tension is unity, the pull of the surface film on the plane $= 2\pi r =$ the hydrostatic pressure on one side of the plane $= p\pi r^2$, where p is the pressure. Since $p\pi r^2 = 2\pi r$, $p = \frac{2\pi r}{\pi r^2} = \frac{2}{r}$, hence when r is small p is large, or when the radius is small the pressure is large. Thus, the pressure is greater in small drops when the surface tension remains the same. But it was shown above that the surface tension is greater the smaller the drop (the greater the curvature). Hence, the pressure is increased in two ways.

Now suppose the surface film of a drop of liquid that floats in another liquid of equal osmotic pressure to be semipermeable. The surface tension of the drop causes a hydrostatic pressure in its interior and pure water will be squeezed out through the surface film until the increase in osmotic pressure equals and replaces the hydrostatic pressure due to surface tension. Hence surface tension increases osmotic pressure.

Without knowing the surface tension of protoplasm we cannot estimate the effect of surface tension on the osmotic pressure of cells. Quincke assumed that the surface tension of protoplasm is the difference between the surface tension of water and that of a certain albumin solution. From this assumption the osmotic pressure of Micrococcus progrediens (diameter = .16 mm) would be 5.75 atmospheres greater than the medium, without taking into account the increase in surface tension due to convexity. This effect decreases rapidly with the diameter of the cell, for one of .02 mm diameter being only .046 atmospheres.

Surface Tension of Aqueous Solutions

As a rule, inorganic neutral salts, and many sugars, very slightly raise the surface tension, whereas acids, bases and most organic substances lower the surface tension. The lowering is not proportional to the concentration, but when a small amount of the substance lowers the tension a certain degree, each addition of the same amount has less and less effect, Fig. 24.

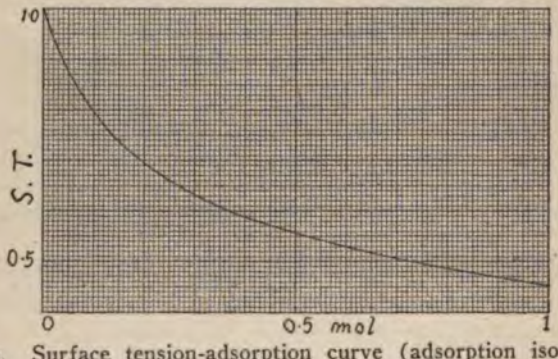


FIG. 24. Surface tension-adsorption curve (adsorption isotherm) of butyric acid (from a class experiment). The surface tension relative to pure water is plotted on the ordinates and the mol concentration of the butyric acid on the abscissae.

A curve similar to Fig. 24, which is the typical surface tension adsorption or adsorption isotherm curve was first figured by Dupré (1866). The necessary consequence of the shape of this curve is that the solute reducing the surface tension is adsorbed. This relation, demonstrated by Dupré and formulated a few years later by Gibbs, was first called to the attention of physiologists through the comparatively recent writings of Wilhelm Ostwald.

The following table gives the surface tension at 15° of .25 molecular solutions of some non-electrolytes, according to Traube. It may be seen that sugars and amino acids raise the surface tension, urea is inactive, trihydric alcohols and amides lower it slightly, and monohydric alcohols, ketones, nitrils, and organic acids and their esters lower it very much. In homologous series the effect is greater the longer the carbon chain. According to Traube it requires only one-third the molecular concentration of one member of the series to lower the tension as much as the preceding member.

.25 mol	s. t. at 15°
Cane sugar	1.007
Grape sugar Mannite	1.004
Glycocoll	1.003
Water	1
Urea	1
Glycerine	.998
Glycol	.99
Acetamid	.986
Methyl alcohol	.966
Acetic acid	.961
Aceto nitrile	.95
Ethyl alcohol	.92
Acetone	.88
Ethyl urethane	.87
Propionitrile	.842
Propionic acid	.83
Methyl acetate	.83
Propyl alcohol	.8
Methyl ethyl ketone	.797
Diethylamine	.75
Piperidine	.738
Ethyl ether	.73
Pyridine	.73
Paraldehyde	.7
Chloral hydrate	.695
Butyric acid	.67
Isobutyl alcohol	.61
Tert. Amyl alcohol	.60
Ethyl acetate	.58—.688
Isoamyl alcohol	.42

Although small amounts of some substances may reduce the surface tension of water to less than half, no substance raises it very much. The explanation is that the substance which lowers the surface tension becomes very concentrated in the surface film and has its maximal effect, whereas the substance which raises the surface tension is driven from the surface, consequently reducing its effect.

The salts of mineral acids and alkalis slightly raise the surface tension whereas HCl and HNO_3 lower it. An electrolyte brings with it the added effects of the ions and molecules.

Surface tension is reduced by the colloids from organisms, but since we do not know their molecular weight we cannot compare them quantitatively with other organic substances. The colloid concentrated in the surface film becomes very viscous, finally forming a membrane insoluble in water. These so-called haptogen membranes form on the surface between water and air, ether, carbon bisulphide, chloroform and many solids. By shaking the solution the colloid may be precipitated in the form of torn membranes, which assume a stringy appearance. In this way enzymes may be inactivated, being either colloidal or precipitated with colloid impurities. The presence of colloids makes an emulsion of oil and water permanent by forming haptogen membranes around the oil drops. It is the haptogen membranes on fat globules in milk that keeps it permanently emulsified, but these membranes seem to be different from caseinogen.

Measurement of surface tension is a convenient method of quantitative chemical analysis provided that only one substance greatly affecting the surface tension be variable in concentration. It is only necessary to plot the graph of surface tension and concentration in order to make a table for converting surface tension readings into concentrations. In mixtures, however, the problem may be more difficult.

G. D. Allen (1916) measured the surface tension of urine containing different concentrations of bile salts. Bile salts greatly increase the surface tension, but NaCl, which in pure solution slightly raises the surface tension, increases the effect of bile salts in lowering the surface tension. H ions increase the effect and OH ions decrease the effect of bile salts. Sodium taurocholate is slightly less effective than sodium glycocholate and, hence, a variation in the proportions of the two salts would slightly affect the result. The following table shows the limits of error when the urine is diluted to a specified specific gravity in order approximately to regulate the concentration of NaCl and other interfering substances.

Showing the Amounts of Sodium Glycocholate Required to Give Any Observed Surface Tension in a Sample of Urine Diluted to a Standard Sp. Gr. of 1.010.

S. T. of sample diluted tt 1.010 sp. gr.	Minimum of sodium glycocholate which may give observed S. T. (Sample is acid.) Diluted sample.	Maximum of sodium glycocholate which may give observed S. T. (Sample is alkaline.) Diluted sample.
Per cent of that of distilled water.		
	gm. per 100 cc.	*gm. per 100 cc.*
95	0	0.001
90	0	0.004
85	0.001	0.008
80	0.003	0.015
75	0.007	0.03
70	0.015	0.065
65	0.033	0.1+
60	0.1+	0.1+

Billard and Bruyant (1905) observed the use made of surface tension by the beetles, Stenus tarsalis and cicendeloides, that dart about on the surface of water. They are pulled forward by the surface tension of the water in front, while the surface tension of the water behind them is decreased by substances discharged from the anus. A model of this may be made by attaching a piece of camphor to one end of a stick floated on water. Traces of the camphor dissolve and lower the surface tension behind, and the stick moves forward. An irregular piece of camphor alone darts about owing to unequal solution on different sides.

Adsorption

As a rule, substances which lower the tension of the water-air surface also lower the surface tension between water and a solid, but the latter can be measured only in terms of adsorption. Traube gives the following data showing the effect of .25 molecular solutions on the tension of the water-air surface as compared with the amount adsorbed by a unit quantity of animal charcoal.

Solute.	Surface tension.	Adsorption.
Acetic acid	.84	.244
Ethyl alcohol	.85	.136
Propionic acid	.7575	.366
Butyric acid	.613	.573
i-Amyl alcohol	.374	.786

It may be seen that surface tension and adsorption are inversely proportional except in the case of acetic acid, which is adsorbed by charcoal more than we would expect from the surface tension of its solution against air. A similar series was obtained of the adsorption of these substances by powdered glass, cotton and silk.

The adsorption curve for different concentrations is similar to the surface tension curve, Fig. 24, a larger proportion of the solute being adsorbed from dilute solutions than from more concentrated solutions. An empirical formula to express this curve that has been suggested is $\frac{x}{m} = ac^{1/n}$, where x is the amount of substance adsorbed, m is the amount of adsorbent powder, c is the concentration of the solution after equilibrium is established and a and n are empirical constants (Freundlich, 1907). N is usually greater than 1 and this makes the equation characteristic for adsorption and surface tension. The equation is not quite correct, however, for concentrated solutions. As the concentration of the solution is increased the surface becomes saturated and will not adsorb any more.

Non-electrolytes that reduce the surface tension compete for a place at the surface. The one which is the more effective in reducing the surface tension will displace the other from the surface. The effect of two or more substances on surface tension is additive if the concentration curve is taken into consideration. The same rules hold for adsorption.

Electrolytes that reduce surface tension are adsorbed. If the surface be electrically polarized, however, it affects the adsorption of electrolytes but not of non-electrolytes. When this is the case, electrolytes and non-electrolytes that reduce the tension do not displace one another on the surface, but act independently.

The adsorbability of anions increases in the order: $SO_4 < Cl < Br, NO_3 < I$, and of cations $Na < K < Rb < Cs$ and $Ca < Sr < Ba$. The ions of heavy metals are adsorbed more than those of lighter metals; the nobler the metal the more this is true.

Neutral salts, such as NaCl, that do not lower the tension of the water-air surface are nevertheless adsorbed by charcoal, paper, etc. The reason for this difference between the adsorption by the air and the solid surface is probably not electrical polarization, since that could not favor the adsorption of both ions of a salt, but is probably due to molecular attraction of the salt by the solid (adhesion). The adsorption of a substance by the air surface is due to the repulsion by the water molecules, but the adsorption by a solid surface might be due either to the repulsion by the water or attraction by the solid (McClendon, 1913 b).

The electric charge of the solid, however, does influence the adsorption of ions. Clay, aluminum hydroxide and ferric hydroxide are usually electropositive, whereas kaolin, mastic, silicic acid, sulphur, charcoal, glass, silk, cotton, wool, starch grains and many other substances are negative. Acid dyes are in general adsorbed by clay but not at all by kaolin, the converse being true of basic dyes.

The electric charge of solid particles may be due to (first) electrolytic solution tension in the case of particles of metal, or (second) to electrolytic dissociation in the case of silicic acid which dissociates H ions or sulphur which partially oxidizes and dissociates H ions, or (third) to absorption in case the particle absorbed one ion of an electrolyte and not the other, and often (fourth) to adsorption of ions. The ions that are responsible for the electric charge of all particles are not known, but H ions may be displaced from positive particles by organic cations.

The electric charge of solid particles may be increased, decreased or reversed by the adsorption of ions. H ions and heavy metal ions make particles more positive, whereas OH ions make them more negative. In the neutralization of the charge of particles, bivalent ions are more than twice as effective as monovalent ions and trivalent ions are much more than three times as effective. This is what we should expect from the curve of adsorption (Fig. 24), since two molecules are not twice as effective or three molecules three times as effective as one molecule.

After reversing the charge of particles their adsorbing power is reversed. Wool will take up very little acid dye until it is made electropositive by the addition of free acid.

CHAPTER VI

ELECTROLYTES, NON-ELECTROLYTES AND COLLOIDS

Since electrolytes in dilute aqueous solution are usually largely dissociated, it is with the ions that we have most to do. Many of the ions are elements and their properties may be predicted from their places in the periodic system. In Fig. 1, curve III, the atomic weights of the elements are plotted on the abscissae and the atomic volumes on the ordinates. There are a number of U-shaped curves. At the tops of the arms are, Li, Na, K, Rb and Cs. These are the alkali metals and are arranged in order of their properties. The first spaces going down the left hand slopes indicate F, Cl, Br and I. These are the halogens arranged in the order of their properties, forming the most important monatomic anions in life processes. We find Be (above B), Mg, Ca, Sr and Ba occupying the first places down the right hand slopes. This is the alkaline earth series. The last three are arranged according to their physiological properties, but Mg sometimes behaves as an alkaline earth metal, sometimes as an alkali metal. It is peculiar also in its physical properties, $MgSO_4$, for instance, being very poorly dissociated. The intermediate elements of the first and second curves, C, N and O and Si, P and S, form many of the compound ions.

The most important anions may be arranged according to their effect on the thermodynamic properties of water as a solvent in the following lyotrope series: CNS, I, (Br, NO_3), Cl, CH_3COO, SO_4, HPO_4, Tartric, Citric. They decrease the compressibility of water from left to right. Those to the left decrease, and those to the right increase the viscosity of water. They increase the saponification of esters by bases from left to right and the reverse is true of the hydrolysis of esters by acids. They increase the surface tension of water from left to right. The electrolytic solution tension increases from left to right. The hydration of NO_3' is small, of Cl' is greater and of SO_4'' is greatest, but the other members do not fall in order, from the available data. Their effect in salting out poorly soluble substances increases from left to right.

The ionic speed of the cations increases in the order: Li, Na, NH_4, K, Rb, Cs (the viscosity of their aqueous solutions decreases in same order), and the order, Mg, Ca, Sr, Ba. They accelerate the hydrolysis of esters by acids from left to right. The reverse is true of the hydrolysis of esters by bases. Solutions of KNO_3, KCl, CsCl and RbCl are less viscous than water. The electrolytie solution tension decreases in the order Li, K, Na and Ba, Ca, Mg, Sr.

The heavy metals are poisonous on account of their low electrolytic solution tension, as will be discussed later.

The H and OH ions are distinguished by their very high ionic speed and relatively low electrolytic solution tensions. They are very active and distinguish the acids and bases.

Non-Electrolytes

The dissociation of the so-called non-electrolytes is so small as not to affect the osmotic pressure. The H ion dissociation and effect on surface tension of many of them has been given. Many of these substances may be salted out of solution by the addition of electrolytes. The order of effectiveness of ions is: $CNS < I, ClO_3, NO_3 < Br < Cl < OH < SO_4 < CO_3$, and $H < Cs < Rb < NH_4 < Li$ (hydr.) $< K < Na$. The same holds true for salting out dissolved gases, which may be included under the non-electrolytes.

Many electrolytes and colloids may be precipitated from their water solutions by non-electrolytes. According to one view this is the substitution of one solvent for another. Salt is more soluble in water than in alcohol, and when alcohol is added to its saturated aqueous solution some salt is precipitated.

According to Traube the precipitating power of non-electrolytes is proportional to their effectiveness in lowering the surface tension of water. The following table gives their power of precipitating Li_2CO_3 as compared with the surface tension of their .25 mol solutions at 15°:

Non-electrolyte.	Ppt. power	Surface tension (.25 mol).
Mannite	—10.7	1.004
Dextrose	— 6.6	1.004
Water		1
Urea	+ 4.6	1
Glycerol	+ 9.4	.998
Glycol	+14.2	.99
Methyl alcohol	+19.8	.966
Ethyl alcohol	+33.7	.92
Acetonitrile	+34.5	.95
Urethane	+41	.87
Propyl alcohol	+41.8	.8
Acetone	+42.5	88
Pyridine	+44.5	.73
Piperidine	+50.6	.738
Ethyl ether	+52.2	.73
Diethylamine	+56.1	.75
Tert. Amyl alcohol	+63.1	.6

Colloids

Although Graham defined colloids as a class of non-diffusible, non-crystallizable substances, Von Viemarn claims that any substance may be made colloidal, and hence we should more correctly speak of the ***colloidal state***. Many constituents of cells, however, are practically always in the colloidal state and are conveniently called colloids (emulsoids). Although some proteins may be crystallized, the crystals contain water and water soluble substances and are therefore not so very different from gels. The colloids are divided into suspensoids and emulsoids.

Suspensoids

The suspensoids, or suspension colloids, are ultramicroscopic suspensions. The particles are said to be liquid when first formed, but some solidify later. With the ultramicroscope the particles may be seen and their size estimated. The ultramicroscope is merely a microscope with a very powerful dark field illumination. The colloidal particles look like stars in the night except that they may appear colored, depending on their size, the smallest appearing blue. This light dispersed by colloid solutions causes them to opalesce. The light may not only be colored, but polarized as well, a phenomenon named after the discoverer, Tyndall. Even cane sugar and raffinose solutions show the Tyndall effect (De Bruyn, 1900).

Whereas with the directly illuminated microscope particles .14 μ in diameter may be seen, with the ultramicroscope we may see particles .004 μ in diameter. Since a molecule of soluble starch is calculated to be .005 μ in diameter, the limit of vision is within the size of the largest molecules. The colloidal particles visible with the ultramicroscope are called submicrons, but particles too small to see singly, amicrons, may cause a diffuse light. Since a diffuse light is caused also by submicrons which are out of focus, their presence must be excluded in order to be sure that the light comes from amicrons. Submicrons show the Brownian movement that is sometimes seen in the ordinary microscope when small suspended particles are present. The amplitude of the vibrations is inversely proportional to their size and the viscosity of the medium.

Suspensoid particles bear electric charges in the same way that the particles of a suspension do. The origin of the charges is different in different cases. It may be due to electrolytic solution tension, as in case of the negative sols (colloid solutions) of platinum, gold or iridium. It may be due to electrolytic dissociation as in case of positive sols of metallic hydroxides or basic dyes or negative sols of silicic, stannic or tannic acids, mastix or sulphonic acid dyes. Or it may be due to adsorption of ions as in case of silver iodide sol.

Colloidal particles of different sizes may sometimes be separated by means of the ultrafilter (Bechhold, 1908).

Suspensoids are precipitated by ions or suspensoids of opposite charge. The isoelectric point, the point at which the particles migrate neither to the cathode nor anode, is the point of complete precipitation. Only a small amount of the precipitant is necessary and if more is added the charge on the particles may be reversed and hold them in suspension. The precipitation follows the adsorption curve (Fig. 24). Hence, bivalent ions are more than twice and trivalent much more than three times as effective as monovalent ions, but this seems not to explain entirely the difference. Among ions of the same valence the less the electrolytic solution tension and the greater the ionic speed the greater is the precipitating power. Thus negative sols are more easily precipitated by H and heavy metal ions. Monovalent and bivalent ions may antagonize one another in precipitation.

A precipitating electrolyte is more effective the faster it is added to the sol. The probable explanation is that when the electrolyte is added suddenly some colloidal particles have their charges reversed and attract others, forming larger aggregates, which are more difficult to keep in suspension. If the electrolyte be added slowly, all of the particles have the same charge, which is not reduced enough to cause precipitation. Evidently the effect of increasing the valence of the ions is the same as the effect of adding them suddenly.

Some precipitating electrolytes partially hydrolyze, forming colloidal solutions of opposite sign to the one to be precipitated. We therefore get mixed effects of electrolytic and colloidal precipitation. Thus, a negative mastix solution is precipitated by ferric chloride and also by ferric hydroxide. If a more concentrated solution of ferric chloride be added to the mastix we get precipitation by the ferric ions. If a very dilute solution of ferric chloride is added, colloidal ferric hydrate is formed by hydrolysis, but precipitation does not occur unless the sum of the charges on the hydrate exactly neutralizes the sum of the charges on the mastix. Hence the precipitating concentration is very exactly defined and varies with the concentration of the mastix sol. Since there are two concentrations of the precipitant that are effective this is called an irregular series.

Low concentrations of non-electrolytes do not precipitate.

The suspensoids are similar to suspensions in that they are precipitated by small amounts of electrolytes and the precipitate is irreversible. They do not affect the viscosity or surface tension of water, or exert osmotic pressure. This is true of dilute sols, but when very concentrated they form gels, the viscosity of which is necessarily high.

True suspensoids are probably not important in cell chemistry, but emulsoids may be changed by heat or reagents so as to assume more or less the character of suspensoids. Emulsoids so treated are said to be denatured. Thus, if a very dilute solution of egg albumin is boiled it is converted into an opalescent solution in which the particles may be seen with the ultramicroscope, and irreversibly precipitated by electrolytes. The denatured albumin particles differ from true suspensoid particles in that they contain water, but not as much as emulsoid particles. Hydroxides (such as dialyzed iron) contain some water.

Living cells may be so changed as to resemble the particles of a suspension or suspensoid sol, although they are probably always killed in the process. The process of agglutination of bacteria consists of two stages: first, the transformation of the bacteria into suspensoid particles by treatment with specific agglutinins or by boiling; second, the precipitation of these particles by electrolytes. The precipitation of a suspensoid sol is always inaugurated by clumping of the particles.

Cholesterin and uric acid may form suspensoid sols and gels. Cholesterin appears to form a true solution in the bile, however, and it cannot be detected within the red blood corpuscles by means of the ultramicroscope.

Lecithin when rubbed up with water forms a sol which is intermediate between a suspensoid and an emulsoid. It resembles an emulsoid in that the particles contain water. It resembles a suspensoid in that the particles may be easily seen with the ultramicroscope. Within the red blood corpuscles, however, the lecithin cannot be seen with the ultramicroscope and evidently forms part of the emulsoid gel, either due to the difference in the medium or to the fact that it is chemically combined with one or more of the other substances present.

Emulsoids

The emulsoids are called hydrophile colloids by Perrin because of their great affinity for water. They are the true colloids, or glue-like substances, according to Graham's definition, except for the fact that some of them may be crystallized with more or less difficulty. When a dry mass of colloid substance is placed in water it absorbs water and swells, with the evolution of heat, forming a gelatinous mass. It usually requires mechanical or other assistance, however, to get it into solution. The colloid particles in sols contain water and the water between them contains some dissolved colloid. It is probably for this reason that the particles do not appear distinct with the ultramicroscope. An absolutely pure solution of gelatine appears clear with the ultramicroscope except at certain concentrations and temperatures, and if some particles are seen in it they probably are impurities. Under certain conditions, however, the particles lose some of their water and approach in character the suspensoids, becoming optically more distinct. Similarly, in an absolutely pure gelatine jelly or gel, no structure can be seen with the ultramicroscope except at certain concentrations and temperatures, whereas in a suspensoid gel the arrangement of particles into chains and the chains into a network may be distinguished. According to Zsigmondy (1912), ½ per cent gelatine shows submicrons on cooling, first mobile but becoming motionless during gelation. The evidence for a structure in a gel is the sudden increase in elasticity during gelation. A gel is not merely a very viscid sol, because agents that increase viscosity induce solation. Fibrin gel seems to be a mass of crystals (W. H. Howell, 1914).

The emulsoids are probably to be regarded as true solutions, in which the molecules are very large and sometimes aggregated into masses that include more or less water. Some colloids probably have a stronger tendency to aggregate than others. Hemoglobin seems to have little of this tendency when in not too concentrated a solution. Hüfner and Gansser (1907) conclude that it is in true solution. If it be assumed that one molecule of hemoglobin contains one atom of Fe and binds one molecule of CO, its molecular weight is about 16700. Calculated from the osmotic pressure of pure solutions, the molecular weight of horse hemoglobin is 15115, and of ox hemoglobin 16321. Since one molecule of hemoglobin cannot contain less than one atom of iron, the molecules must be separated in solution in order to exert so high an osmotic pressure.

Bechhold (1908) has attempted to estimate the size of the particles in emulsoid sols by forcing them through ultrafilters of different grades. These filters are made by impregnating filter paper with an emulsoid sol and hardening it. The size of the pores is determined by the concentration of the sol before being hardened. There are two difficulties with the method: first, the filter may adsorb the colloid, second, some water may be pressed out of the sol in the filter and increase its concentration with enormous increase in viscosity until the flow is stopped. Notwithstanding these difficulties Bechhold has arranged emulsoids in the following series beginning with those with the largest particles:

Caseinogen (in milk)
Gelatine (1 per cent)
Hemoglobin (1 per cent)
Serum albumin
Protalbumoses
Duteroalbumoses A
Deuteroalbumoses B
Dextrin

Emulsoids enormously increase the viscosity of water. One per cent gelatine increases the viscosity of water 29 per cent.

Emulsoids reduce the surface tension and are therefore adsorbed. They become denatured in the surface film to the extent that they no longer go into solution in some cases (haptogen membranes). In this way they may be precipitated by shaking the solutions. The surface films are submerged and distorted, forming stringy masses, while new surface films are formed. Emulsoids adsorbed by solids cannot be washed out with water because they have become insoluble. Emulsions of oil and water are permanent if colloids are present as the haptogen membranes formed about the oil drops prevent them from fusing. Soap acts in this way. Some colloid from milk glands forms haptogen membranes around the fat droplets, but it is said not to be caseinogen. The plasma membrane on the surface of protoplasm is supposed to be of this nature although the colloids in its composition have not been ascertained. Presumably, the colloids of the cell: proteids, lecithins, and cholesterin, enter into its composition.

Emulsoids are not precipitated by small amounts of electrolytes unless they have been denatured. They may be salted out by large amounts of neutral salts in the same way that truly dissolved substances, such as gases or alcohol, may be salted out of their aqueous solutions. The electric charge of the colloid is not entirely without significance in the salting out process, however, as will be seen in the anionic series. The order of effectiveness of anions in salting out soluble non-electrolytes is (beginning with the least effective): CNS, I $<NO_3$ $<Cl$ $<CH_3COO$ $<SO_4$ <Tartrate <Citrate (Hofmeister, 1891). The order of effectiveness with negative emulsoids is the same,

but the order is reversed in case of positive emulsoids. The order of effectiveness of cations in precipitating negative emulsoids is Li $<$Na $<$K $<$Rb $<$Cs, and with positive emulsoids the reverse.

There is not much difference in the precipitating power of the ions of alkali metals, but the bivalent cations (except Mg) precipitate emulsoids much more effectively than monovalent cations, and trivalent cations more effectively than bivalent. The effectiveness of the heavy metals and sometimes Ca, Sr, Ba and $H^{\cdot}$ in precipitating emulsoids lies partly in the fact that they denature the emulsoids, making them easier to precipitate. Such precipitates are irreversible, whereas the precipitations with neutral salts of the alkali series are reversible.

Neutral salts reduce the osmotic pressure of emulsoid solutions, presumably by causing the particles to aggregate and form larger ones, thus reducing the number. The order of effectiveness of the anions is the same as given above.

Emulsoids are precipitated by some non-electrolytes, such as alcohol (see non-electrolytes).

Many of the emulsoids are proteids and are amphoteric. These are made electropositive by acid and negative by alkali. There is probably a chemical reaction of the nature: $Alb{\cdot}OH+HCl=Alb{\cdot}Cl+HOH$, after which the acid albumin dissociates into $Alb^{\cdot}$ and Cl'; and $Alb{\cdot}H+KOH=Alb{\cdot}K+HOH$, after which the alkali albuminate dissociates into Alb' and $K^{\cdot}$.

Small amounts of acid or alkali increase the osmotic pressure and viscosity, decreasing the surface tension of albumin solutions. This is probably due to the fact that the acid and alkali albuminates are more soluble or highly hydrated in the sense that the molecules are not aggregated and hence the "molecular concentration" is increased. The addition of larger amounts of acid increases the effect up to a certain limit, above which the albumin is denatured.

Since the proteins are amphoteric they dissociate both H and OH ions, and are either positive or negative according to whether they dissociate more OH or more H ions. According to the law of mass action, the greater the concentration of the H ions in the solvent the less H ions will be dissociated, and the greater the concentration of OH ions (or the less the concentration of

H ions) in the solution the less will be the OH ion dissociation by the protein. Therefore, at a certain H ion concentration of the solvent the $H^{\cdot}$ and OH' dissociation of the protein will be equal, and the protein will migrate neither to the cathode nor to the anode in an electric field. This is called the isoelectric point of the protein, at which the protein can be most easily precipitated (by alcohol, for instance). At this point the viscosity of the sol is at its minimum.

The albumins, globulins and some other proteins are more acid than basic. The isoelectric points of some of the proteins as determined by Michaelis (1912) are as follows:

Protein	PH
Serum albumin	4.7
Serum globulin	5.4
Casein	4.7
Gliadin	9.3
Edestin	7.
Oxyhemoglobin	6.74
Pancreasnucleoprotein (trypsin)	3.52
From typhus bacilli	4.4
From paratyphus bacilli	4.

The isoelectric point of sols is also the point of least hydration, viscosity and stability, and of greatest surface tension. The size of the colloid particles is probably largest at this point.

In the precipitation of emulsoids as well as suspensoids, monovalent and bivalent cations are antagonistic to one another.

Emulsoids are precipitated by suspensoids, but if too much of the emulsoid is present for precipitation it forms coatings on the suspensoid particles and prevents their being precipitated by small amounts of electrolytes. In order to obtain complete precipitation, the suspensoid must be of opposite electric charge to the emulsoid.

Emulsoids may precipitate one another even when of the same sign electrically. Thus nucleic acid precipitates other proteins and specific precipitins precipitate the precipitable substances, both being of the same sign (cf. Teague and Buxton, 1907). According to Michaelis, the ratio between precipitin and precipitable substance in the precipitate is not constant, and is not therefore a chemical reaction, but most probably an adsorption phenomenon.

The time element in colloid phenomena is little understood. A less concentration of the precipitant is necessary if it be added suddenly than if added gradually. A less amount of antitoxin is necessary to render a toxin solution inert when added at once, than when added gradually. On the other hand, time is favorable to the increase of viscosity. Gels become firmer with age and haptogen membranes likewise, even though perfectly protected from evaporation. This phenomenon is known as hysteresis or secondary change.

The gels may be considered very viscous sols but the cause of the great viscosity probably lies in the supposed structure. The swelling of gels or gelatine plates may be made to produce a pressure which is thought, by some authors, to be analogous to the osmotic pressure of sols. The pressure is increased by acids and alkalis. The emulsoid gel may be converted into a suspensoid gel in the same way that an emulsoid sol is converted into a suspensoid sol by coagulative agents. Some sulphates and tartrates cause gelatine gels to shrink, bromides and nitrates causing them to swell (Hofmeister, 1888). According to Lenke (1916) these two groups differ in that with the first there is an optimum concentration for swelling and with the second the greater the concentration the greater the swelling. Their effect on gelation is the inverse of their effect on swelling, the series being: $SCN < Br, NO_3 < Cl < CH_3COO < \text{Cit.} < \text{Tart.} < SO_4$. Glycerine, dextrose and saccharose increase the viscosity of gelatine and starch gels, and urea decreases it. The reverse is true of their effect on swelling.

The ionic series which holds for negative emulsoids and is reversed for positive, becomes irregular as we near the isoelectric point. This explains the variations in the ionic series found in various physiological experiments since the emulsoids in living cells are near the isoelectric point.

According to Pauli, anions increase swelling of gelatine gels and cations decrease it, the greater the atomic weight of the cation, the greater its effect.

Traube (1915) claims that the more a substance hastens solation (solution of a gel), the more it favors the rate and amount of swelling, the more it hastens gelation, the more it disfavors the rate and amount of swelling. He finds that the stronger acids hasten gelation when very dilute (PH = isoelectric point) and that there is a maximum retardation at about .02 n at which Fischer observed the maximum swelling, and Pauli and Wagner (1910) observed the maximum viscosity (of serum albumin in the last case). Traube claims that $Ca^{\cdot\cdot}$ and $K^{\cdot}$ hasten gelation and $Na^{\cdot}$ retards it and therefore $Ca^{\cdot\cdot}$ antagonizes $Na^{\cdot}$. He claims that anesthetics retard gelation (see Chapter XI).

Chiari (1911) found the least swelling of gelatine to occur when the PH = 4.7, which corresponds closely to the isoelectric point, PH = 4.6 as determined by Michaelis. No other investigator of the effect of acids and alkalis on emulsoids has determined the PH.

CHAPTER VII

ENZYME ACTION

Enzymes are classified according to their action, and not according to their constitution. Although Berzelius defined catalyzers as those substances which by their mere presence cause reactions to take place between other substances, many catalyzers are now known to take part in the reactions. Ostwald supposed that catalyzers accelerate the rate of only those reactions which go on at a slower rate in the absence of the catalyzer. It is true in certain cases, however, that the catalyzer may change the point of equilibrium of a reversible reaction. An example of this was observed by Bodenstein and Dietz (1906) in the reversible decomposition and formation of amyl butyrate. In the presence of an excess of water and amyl alcohol, the same equilibrium point was reached whether the amyl butyrate or the amyl alcohol and butyric acid were present in the beginning. But if pancreas lipase were used as a catalyzer, 75 per cent of the ester was formed or left against 85 per cent of the ester when HCl was used as a catalyzer.

The chief distinction that is supposed to separate enzymes from other catalyzers is their specificity. This is perhaps overrated, but is fairly well established in some cases. Amygdalin may be decomposed by means of emulsin into HCN, benzaldehyde and dextrose. Emmerling (1901) showed that this took place in three stages, each of which was accelerated by a different enzyme, and that all three enzymes were present in emulsin. The reversal of the process could be brought about by applying the enzymes in a certain order to the separate stages.

In case of stereoisomeres or optically active isomeres, an enzyme may accelerate a reaction involving one isomere more than the other. If HCN and benzaldehyde are mixed in solution optically inactive benzaldehydecyanhydrin is formed, but if the reaction is accelerated by means of emulsin, d-benzaldehydecyanhydrin is formed. E. Fischer (1898) observed that beta-methyl-d-glucoside is split by emulsin whereas alphamethyl-d-glucoside is not.

This form of specificity is not peculiar to enzymes. According to Bredig and Fajans (1908) the splitting of camphocarbonic acid into camphor and CO_2 is accelerated by certain organic compounds. Natural nicotine decomposes d-camphocarbonic acid 12 per cent faster than l-isomere. Chinin decomposed the l-isomere 46 per cent faster than the d-isomere.

The specificity just mentioned is quantitative and not qualitative, whereas the specificity of enzymes is supposed to be qualitative. It was shown by H. D. Dakin (1905), however, that the specificity of lipase for certain d-esters in preference to the isomeric l-esters, is quantitative.

More unexpected than the specificity of enzymes, is the change in specificity on reversing the reaction. A. Croft Hill (1898) succeeded in synthesizing a disaccharide from dextrose by means of maltase from yeast. Emmerling (1901) showed that this disaccharide is not maltose, as was to be expected, but isomaltose. E. F. Armstrong (1903) continued this investigation and found that:

> whereas maltose is split into glucose by maltase
> glucose is synthesized to maltose by emulsin, and
> whereas isomaltose is split into glucose by emulsin
> glucose is synthesized to isomaltose by maltase.

The explanation of this anomaly is that the enzymes change the point of equilibrium of these reversible reactions. In a mixture of maltose and glucose, maltase displaces the equilibrium toward the side of the glucose, whereas emulsin displaces it toward the side of the maltose. If HCl is used as a catalyzer, when equilibrium is established glucose, maltose and isomaltose are present in the same proportions, irrespective of the first used. If glucose only is present at the start, and maltase is used, very much more isomaltose than maltose is formed, but if emulsin is used, much more maltose than isomaltose.

Since enzymes merely accelerate reactions that would take place without them and all reactions are theoretically reversible, the synthesis of complex bodies from their decomposition products might be expected with the aid of the same enzymes that affected the decomposition. Hill was the first to accomplish synthesis with enzymes, and obtained a different compound from the original. Kastle and Loevenhart (1900) succeeded in synthesizing a fat, ethyl-butyrate, from butyric acid and alcohol, by means of lipase of the pancreas. The attempts to synthesize proteins have not given such unequivocal results. Since the chemical constitution of proteins is unknown, it is not surprising that some difference of opinion should arise in the interpretation of changes occurring in mixtures of protein decomposition products and protease. Some interpret the appearance of a precipitate as synthesized protein, others as coagulation of albumoses already present. Perhaps one difficulty in producing syntheses is that they are likely to be endothermic reactions, which absorb energy from without, as in case of the synthesis of carbohydrates in the chloroplast of the green plant under the influence of sunlight. The energy of the light is absorbed during the synthesis. In this process the CO_2 is reduced and hydrated with the formation apparently of formaldehyde, which is synthesized into sugar and starch.

As was previously stated, the important effect of a catalyzer is on the rate of a reaction. In case the concentration of the catalyzer remains constant, implying that if it enters into the reaction, a vanishing quantity of it is bound at any one time, the rate of the reaction may follow the law of mass action for a monomolecular reaction.

$$-\frac{dx}{dt} = c\ (a-x)$$

In which $a-x$ is the concentration of the substance at any particular moment, and c is the constant denoting the speed of reaction. This law holds true, for instance, for the hydrolysis of cane sugar by means of a fixed H ion concentration, since the concentration of the H_2O that enters into the reaction remains the same, and the only variable is the concentration of the cane sugar. If the concentration of the H ions is varied, the reaction constant, c, varies in the same ratio.

One of the oldest theories of the mechanism of catalytic action is that the catalyzer enters into the reaction transforming it into two stages, the sum of which takes less time than the uncatalyzed reaction. In this way is explained the action of nitrous oxide in the oxidation of sulphurous acid, and the action of sulphuric acid in the conversion of alcohol into ether.

The catalyzer may change the course of the reaction, as in the observation of Slator (1903) on the chlorination of benzol, in which the Cl may be substituted or simply added. Different catalyzers favor one or the other form of combination. This is illustrated in enzymes by the difference in the form of proteolysis produced by pepsin and by trypsin.

In case of reactions in a heterogeneous system, of which a diphasic system is the simplest case, not only the rate of the chemical reaction but also the rate of diffusion may enter into the rate of transformation of the substance.

In the first case, the second phase may be a better medium for the reaction than the first phase, and so act as a catalyzer. Bredig (1901) showed that methyl acetate dissolved in benzol is not saponified, even with the addition of triethylamine. If the mixture is shaken with 2.5 per cent water to form an emulsion, saponification takes place with great rapidity, because the water dissociates the triethylamine, and the resulting OH ions catalyze the saponification of the methyl acetate. The same principle seems to be involved in the catalysis of the union of hydrogen and oxygen by means of palladium or other noble metals. The palladium dissolves these gases, forming a so-called solid solution. Hoitsema (1895) has shown that the amount of hydrogen dissolved by the palladium is proportional to the square root of the hydrogen pressure, and the hydrogen, therefore, does not exist in the metal as H_2 but as H. If this atomic hydrogen is more active than molecular hydrogen, the catalytic action of the palladium is explained.

The second phase of a diphasic system may increase the rate of reaction by adsorption. Bayliss (1915 b) has shown that enzymes may act in a medium in which they are insoluble. We shall consider this aspect of the subject under the head of oxidation (Chapter XV).

In case the rapidity of the chemical reaction in or on the second phase is very great, perhaps too great to measure, the rate of transformation is determined by the rate of diffusion in the first phase toward the second. This seems to be the case with many reactions catalyzed by finely divided platinum and is supposed to be a factor in enzyme reactions, since enzymes seem to be of colloidal nature.

Catalysis with colloidal solutions of precious metals resembles the action of enzymes in many ways. Both have a temperature optimum, that is, both are rendered inactive by boiling and even lower temperatures. According to Ernst (1901) the temperature optimum for the oxidation of hydrogen under the influence of platinum sol lies between 65° and 85°. Both are affected by the reaction of the medium. The decomposition of H_2O_2, either by platinum sol or by the enzyme, catalase, is favored by a slightly alkaline reaction. Both the catalase and oxidase action of enzymes or colloidal precious metals is suspended by HCN and returns when the HCN is removed, if just enough were used to suspend action. The same is true of the action of H_2S. On the sols of Cu and Fe, however, HCN increases catalytic activity, according to Kastle and Loevenhart (1903). Finally, Meyerhof (1914 b) has shown that the action of platinum sol on H_2O_2 is reduced by the presence of anesthetics, in the same way that enzyme actions are reduced by anesthetics. The anesthetic forms a protective film on the platinum particles, due to adsorption.

The importance of the reaction of the medium, that is, the H ion concentration, on the activity of enzymes has long been known. Michaelis (1914) attempts to explain this action by assuming the enzymes to be weak electrolytes, i. e., acids, bases or ampholytes, whose dissociation is affected by the H ions. At the optimal H ion concentration the activity of the enzyme solution is proportional to the concentration of the enzyme and all of the enzyme is in the active form. At any other H ion concentration some fraction of the enzyme is in an inactive form, and therefore the total activity is reduced.

According to Sörensen (1909) and Michaelis and Davidsohn (1911) the optimal PH for the invertase of yeast is about 4.3. Above the optimum the activity of the enzyme decreases to zero at about 9, whereas below it the activity decreases (because the enzyme is destroyed?) so that little action is obtained below 3 or 2, depending on the duration of the experiment. Michaelis interprets this as follows: The enzyme is an ampholyte with the isoelectric point at the optimal H ion concentration. Hence, it is the undissociated molecules of the invertase that are active. Above the isoelectric point (alkaline side) the invertase dissociates as an acid, and the activity curve corresponds to the curve for the undissociated part of an acid. Below the isoelectric point (acid side) the invertase dissociates as a base, but the invertase cations are very unstable and soon become denatured, so that the activity of the enzyme is not restored by return to the isoelectric point.

In the same way Michaelis concludes that trypsin and erepsin are acids whose anions are proteolytic. Whether the theory of Michaelis is right or wrong, the relation of the H ion concentration to the activity of enzymes is no less important. The following table gives the optimal H ion concentration for the action of several enzymes (McClendon, 1916 b).

Enzyme	Optimum PH.
Amylase, ptyalin	6.7
Catalase of blood and liver	7
Cellase	6.
Glycolase of blood	7.52
" (Invertase)	4.5
Maltase (maltose)	6.6 (3 at 35°, 7.2 at 47°)
" (alphamethylglucoside	6.2
Inulase	3.
Lipase of stomach	6.7
" " Ricinus	3.
" " pancreas and blood	8
Oxidase, laccase	7.
Protease, erepsin	7.8
" pepsin	1.5—2
" " or rennin (plastein formation)	1
" trypsin (peptone)	7.7
" " (gelatine)	9.7
" bacterial	7.2
" of malt	4.5
" rennin	5.7
Urease (comb. with urea)	8.7
" (decomposition)	7.

The optimum PH for enzyme action is of interest in connection with the reaction of the parts of the alimentary tract, where the digestive enzymes act.

The saliva is generally supposed to be alkaline, but according to Michaelis (1914) it has a PH of about 6.87, which is near the optimum for the action of ptyalin (6.7).

The optimum PH for pepsin is, according to Sörensen (1909), 1.5-2, and the results of Michaelis and Davidsohn fall between these limits. Sörensen observed considerable digestion at PH = 0.7 near the maximum acidity and PH = 5 near the minimum acidity. From curves showing the rise in acidity of the stomach measured with the hydrogen electrode (McClendon, 1915 e) it may be observed that the acidity becomes high enough for digestion to commence during the first hour and the optimum is reached by about the end of the second hour if at all. Menten (1915) found the PH of pure human gastric juice to vary from 0.92 to 1.58, corresponding to an average of .36 per cent HCl. Apparently the reaction of the stomach is determined by the amount of food in it and the rate of flow of the juice. The juice secreted into an empty stomach would have a very high acidity, but Boldyreff (1915) has shown that it is lowered by regurgitation of pancreatic juice. The PH in hyperacidity, from the figures given by Michaelis (1914), indicates not nearly so high an acidity as the pure gastric juice. It seems probable that hyperacidity is brought about by closure (spasm) of the pylorus, preventing removal or neutralization of the acid.

The PH of the pure gastric juice of the infant is about 2.3 (McClendon, 1915 e), being nearly the optimum for the action of pepsin, which it contains, but the juice is apparently secreted so slowly that the PH is 5.3, while the stomach is full of milk, which is about the optimum for the action of rennin (pepsin?) in coagulating milk.

The PH of the adult duodenum is about 7.7 or the optimum for the action of trypsin and erepsin on peptones.

In the infant a very peculiar state of affairs exists. As the milk leaves the stomach the acidity rises until it equals that of the pure gastric juice about four hours after nursing. The neutralizing power of the pancreatic juice is very low, so that the duodenum has about the same reaction as the pylorus. The PH of the duodenal content is usually about 3.1 and pepsin is present, so that peptic digestion must take place in it.

It has been shown by a number of observers that the rate of enzyme action does not always vary in direct ratio with the concentration of the substance to be transformed, substrate, but that high concentrations of the substrate are relatively detrimental.

Many hypotheses have been made to account for this anomaly. An increase in the substrate concentration may increase the viscosity and therefore decrease the speed of diffusion. An increase in the beginning concentration of the substrate means an early accumulation of reaction end products which may be detrimental to the reaction, either by starting a reaction in the reverse direction or by combination with the enzyme. This hypothesis is supported by the fact that the splitting of cane sugar by invertase is retarded more by fructose than by glucose, and mannose or glycerine have about the same inhibiting activity as glucose. It has been supposed that the enzyme may become saturated with the substrate, based on the fact that in some cases the rate of reaction depends on the concentration of the enzyme more than on the concentration of the substrate.

In some cases the products of the reaction activate the enzyme and hence the rate of reaction constantly increases. This type of reaction is called autocatalytic, but it is to be remembered that it does not catalyze itself in the sense of furnishing an enzyme, but increases the rate by activating an enzyme already present. Thus, alcoholic fermentation either with living or dead yeast increases with the accumulation of the end products up to a certain point. Hoyer (1907), in studying the saponification of castor oil during the autolysis of ground castor beans, observed that the reaction proceeded slowly until the H ion concentration reached a certain point, when it suddenly increased. If the H ion concentration were increased at the start by the addition of acids, the splitting of the oil was rapid from the first. The higher fatty acids produced by the splitting of castor oil were probably not sufficiently soluble to produce the required PH alone, and were aided by CO_2 and lactic acid appearing in the mixture. But we may imagine the splitting of the ester of a *lower* fatty acid by the same enzyme, in which the fatty acid produced would cause the rate of reaction to increase. This might be called autocatalysis. According to M. Morse (1916) the autolysis of ground animal tissues is autocatalytic, since the rate of proteolysis is proportional to the PH which constantly increases during the reaction in a characteristic curve. Bradley and Taylor (1916) conclude that H ions act not on the enzyme but on the substrate, increasing the number (and consequently the total surface area) of the colloid particles.

CHAPTER VIII

PERMEABILITY OF CELLS

It was shown by Nägeli, Pfeffer and de Vries that concentrated solutions of neutral salts cause plant cells to shrink by extracting the water from them. The shrinkage, plasmolysis, of plant cells may be easily detected because, though the cell wall does not shrink, the shrinkage of the protoplasm separates it from the cell wall, leaving a space between the two. Solutions with equal plasmolytic power de Vries (1884) called isotonic solutions, and showed a relation between their percentage concentrations and the molecular weights of the solutes. This was the foundation of van't Hoff's theory of osmotic pressure. The discrepancies in the relation between the concentration of isotonic solutions and the molecular weight of the solutes was explained by Arrhenius on the assumption that electrolytic dissociation increases the number of particles in solution. In this way a method was developed of measuring the osmotic pressure of plant cells. The osmotic pressure of solutions that just fail to plasmolyze the cells is equal to the osmotic pressure of the cells. If animal cells are used, some method of determining the first decrease in volume must be used. When the cells are in large masses, changes in volume may be determined by weighing the masses. If the cells are free, as with blood corpuscles, their volume may be determined by centrifuging them in graduated tubes (Hamburger, 1893).

Some substances do not plasmolyze cells no matter how great the concentration, due to the fact that the cells are permeable to the substances which consequently cannot exert any osmotic pressure on the cells. Others cause only a temporary plasmolysis because they slowly penetrate the cells. When the concentration becomes the same outside and inside they cease exerting osmotic pressure.

The question arises as to whether the whole cell substance, or only the surface, is impermeable to salts. The protoplasm of most plant cells forms only a thin surface layer, the primoidal utricle, the interior being filled with cell sap which is an aqueous solution of many substances. Young plant cells, and all animal cells, have only small or no vacuoles filled with cell sap, and little or no nuclear sap, whereas mammalian erythrocytes are entirely composed of a homogeneous gel. In the plant cell, at least, only the surface layer can be the impermeable portion, and Pfeffer found evidence that not all of the primoidal utricle, but only the surface film, is impermeable. Höber showed that only the surface of erythrocytes is impermeable to ions. His methods are complicated, as will be seen from the following paragraphs.

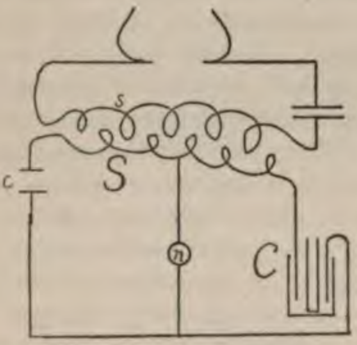


FIG. 25. Scheme for estimating the electric conductivity of cell interiors by the increase in capacity of a condenser into which the cells are introduced (from Höber, 1914).

It was shown by G. N. Stewart that erythrocytes do not conduct the electric current, and are therefore impermeable to ions, unless they are laked. Höber showed that ions move freely in the interior of the erythrocytes notwithstanding the fact that they cannot get in or out. This was first done by the "capacity method" (1910 a). The capacity of an electric condenser is increased if a conducting body is introduced into the dielectric between the two plates of the condenser, the increase being greater the greater the electric conductivity of the body. The apparatus consists of a primary coil (*s*, Fig. 25), through which high frequency electric oscillations are passed, such as are used in wireless telegraphy. These oscillations induce similar ones in the secondary coil, *S*, which is connected at one end with the adjustable condenser, *c*, and at the other with the condenser, *C*, between the plates of which the material to be investigated is placed. The other plates of these condensers are connected, and the circuit bridged by an instrument, *n*, for detecting a current, according to the principle of the Wheatstone bridge. If the capacity of *c* does not equal that of *C* some current will flow through *n*. The plates of *c* are separated by such a distance that no current flows through *n*, which means that the capacities of *c* and *C* are equal. A test tube filled with the erythrocytes is placed between the plates of *C*. If the capacity of *C* is thereby increased, the plates of *c* would have to be approximated in order that no current flow through *n*. In order to prevent error due to the conductivity of the fluid wetting the outside of the corpuscles, they are washed repeatedly with isotonic sugar solution. By filling capsules corresponding in size to the corpuscles with solutions of different conductivity, and finding the conductivity of the solution which causes the same increase in the capacity of *c* as was caused by the blood corpuscles, the conductivity of the interior of the corpuscles may be determined. Höber did not make his capsules of conducting solutions as small as the blood corpuscles nor consider the question of size at all. He calculated that the conductivity of the interior of the corpuscles is between that of a .1 and a .01 normal KCl solution.

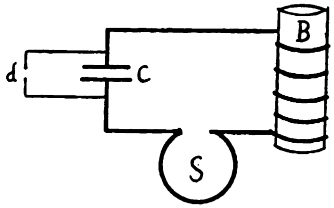


FIG. 26. Scheme for estimating the electric conductivity of cell interiors by the damping of the inductance of a coil in the core of which the cells are placed (from Höber, 1914).

Another method for measuring the internal conductivity is the damping method (Höber, 1915 b). In Fig. 26, *S* is the secondary coil in which high frequency electric oscillations (10^7 a second) are induced, *B* a coil around a beaker, *c* a condenser, and *d* a detector with which to observe any change in the oscillating current. If the beaker is filled with a conducting solution the oscillations are damped, as shown by the detector. If erythrocytes are placed in the beaker the oscillations are damped as much as by 0.4 per cent NaCl solution. This remains the same if the plasma membranes are destroyed by saponin. Since the conductivity, as measured by the Kohlrausch method, is increased ten times by the destruction of the plasma membranes, they seem to be the chief cause of the low conductivity of the normal erythrocytes.

A third method for measuring the internal conductivity of cells is the use of high frequency electric oscillations instead of induction coil currents, in the Kohlrausch method (Höber, 1913 b). When an alternating current is passed through a solution, the cations move in one direction until the current changes, then move in the opposite direction, arriving at their original positions. The anions behave similarly. The distance traveled by an ion is proportional to the duration of one phase of the current, or inversely proportional to the frequency of the alternations. The higher the frequency the less the distance traveled by an ion. When the frequency is ten million per second the distance traveled by an ion is insignificant. The plasma membranes stop very few ions directly, but stop or retard all of them by the electric polarization produced by the stoppage of a few ions. The less the distance that would be traveled by an ion, the less the polarization, hence the very slight polarization when high frequency oscillations are used. The conductivity measured by means of high frequency oscillations is therefore practically the same, whether the plasma membranes are present or absent. It should be noted, while this line of reasoning is in mind, that the conductivity of erythrocytes, measured by the Kohlrausch method, using an induction coil with rapid interrupter, is too high, and it is theoretically only with a direct current and nonpolarizable electrodes that the true conductivity may be measured. In actual practice the errors with the direct current are usually so great that the Kohlrausch method is to be preferred.

By means of the damping method, Höber found the internal conductivity of frog's muscle that had been washed six hours in isotonic sugar solution, to be equal to that of a 0.15 per cent NaCl solution, whereas the Kohlrausch conductivity = 0.03 per cent NaCl. It seems probable, therefore, that the chief impermeable layer of all cells is the plasma membrane, although other membranes, nuclear membranes and vacuole membranes, may also be impermeable to many substances.

These experiments of Höber are selected because the cells are not injured in the least. If space permitted, a large number of experiments which go to show that the plasma membrane is the semipermeable part of the cell could be described. The word semipermeable was first used to describe permeability to water and to nothing else. This is never true of the plasma membrane, since it is always permeable to oxygen, for instance. The word is now used to denote limited permeability. It will be shown later that the plasma membranes of some cells are impermeable to water.

Before considering the permeability of cells to various substances it is well to discuss the diffusion of substances in general, from one phase to another of a diphasic system in particular.

According to Euler the diffusion of undissociated molecules through water is inversely proportional to the square root of the molecular weight, large molecules therefore diffusing more slowly than small ones. The speed of diffusion of ions is given on page 24.

Diffusion is retarded by increase in the viscosity of a solution, hence the emulsoids, which enormously increase the viscosity, decrease diffusion. From this it also follows that those substances that increase the viscosity of water diffuse slowly.

The molecules of a substance in solution move in all directions, the substance diffusing from regions of higher to regions of lower concentration. The rate of diffusion is proportional to the concentration gradient.

In the diffusion of a substance from one phase into another, three processes are involved: first, the diffusion to the phase boundary; second, solution in the second phase; third, diffusion away from the phase boundary.

Substances which lower the surface tension collect at the phase boundary, this step in the process being rapid in this case. This concentration in the surface film is due to two factors, repulsion by the first phase, attraction by the second phase. According to I. Traube, substances which lower the surface tension of water do so because they have a low molecular attraction for water molecules. Molecules which lower the surface tension of water are repelled by the water molecules and tend to collect at the surface of any other phase in the water. But the degree of this collection is also influenced by the attraction of this second phase for the molecules in question. A substance will not diffuse into a second phase in excess of its solubility unless it is removed by combining with some other substance or changed as by dissociation or association.

After a substance enters the second phase, if it lowers the surface tension of this phase it will tend to remain in the surface film rather than to diffuse into the interior. Its diffusion is also influenced by the viscosity of this second phase.

Thus the rate of diffusion of a substance from one phase to another is determined by a number of factors. The process being reversible, these factors determine the partition at equilibrium.

The partition of a solute between two phases may be determined by shaking up the substance with equal volumes of the two phases, then determining the concentration of the substance in the two phases. A number representing the ratio of these two concentrations is called the partition coefficient. Berthelot and Jungfleisch (1872) have shown that the partition coefficient of a substance is not affected by the concentration of the substance, provided it has the same molecular condition in the two phases. If, however, the molecular condition changes in one phase with the concentration, the partition coefficient changes with the concentration. For example: consider the partition of common salt between benzol and water. The partition of the undissociated molecules is the same at all concentrations, but salt is dissociated in water and not in benzol. This means that $Na^{\cdot}$ and Cl' are insoluble in benzol. If the salt is very concentrated in the water there will be a large proportion of undissociated molecules and some of these will pass into the benzol, but if the salt is very dilute all, practically, will be dissociated in the water and as the ions are insoluble in benzol all of them will remain in the water. Thus benzol, which dissolves only traces of salt, will take none from an aqueous solution at infinite dilution.

The plasma membrane is probably a separate phase, and if so, in considering the permeability of a cell we must consider the partition of the solute between the medium and the plasma membrane, as well as between the plasma membrane and the protoplasm. In many cells the protoplasm consists of more than one phase. In the frog's egg, four phases in the cytoplasm and two in the nucleus may be separated by means of the centrifuge (McClendon, 1910 a). The partition of a solute between these phases is therefore to be considered. Some solutes may be in a different molecular condition within the cell, or they may be precipitated or adsorbed to phase boundaries. All of these factors make the subject of cell permeability a very complex one, no general rules without exceptions having been found. All we can do at present is to collect data on the permeability of cells to various substances. The most exact method is the determination of the partition of a substance between water, or some aqueous solution, and the cell, but unfortunately very little of this data has been collected. When a substance is precipitated within a cell, a partition equilibrium may never be reached. If the substance in question is absolutely insoluble in the plasma membrane no diffusion can take place. If the plasma membrane changes with the physiological condition of the cell, substances may become imprisoned in a cell that becomes impermeable to them later. Furthermore, substances may be formed in cells by chemical reactions which will not penetrate cells from the exterior.

Traube attempted to show the ease with which a substance penetrates a cell to be inversely proportional to its molecular attraction to water. Therefore, the more a substance lowers the surface tension of water the more easily it penetrates cells. As was stated above, this is only one of the factors involved. It has been found that substances which very greatly lower the surface tension of water do penetrate cells, but there is not always a constant ratio between the surface tension lowering and the penetration. This same idea championed by Batelli was attacked by Fluzin, who claims that the attraction of the membrane for the solvent is also important. The idea that degree of hydration on the two sides of the membrane influences osmose is also held by H. N. Morse.

Overton supposed plasma membranes to be composed of what he called lipoids. These are cholesterin, lecithin and some other substances that may be extracted from cells with alcohol, and are soluble in ether. Cholesterin is a polyhydric alcohol, crystallizing in waxy plates. Lecithin is a glyceride in which one hydrogen is substituted by phosphoric acid and the other two by fatty acids, one hydrogen of the phosphoric acid being substituted by choline. Lecithine is a waxy substance which swells in water and alters its power of dissolving other substances, finally passing into a colloidal solution. In order to obtain the lipoids in a dry, fluid condition, Overton and others dissolved them in benzol, xylol, toluol, chloroform or oil of turpentine. Many substances which dissolve in one of these mixtures easily also penetrate cells easily, but exceptions are found. The partition coefficients were usually not determined, and a reliable quantitative method of estimating permeability was not always used.

The composition of the plasma membrane remains a mystery. It seems logical to assume that its building stones are selected from the chief constituents of cells, proteins, fats, lecithin, cholesterin, and carbohydrates. It is a very unstable structure as will be shown later.

Some attempts have been made to find or construct membranes with the same properties as the plasma membrane. Pascucci (1907) impregnated silk with lecithin and cholesterin and found this membrane to show some of the properties of the plasma membrane of the erythrocytes. It was shown by A. Brown (1909) that the covering of the barley grain has the same permeability as some cells, but he has not proved that this property is due to the cellulose it contains or to any known substance. Beutner claims to have made membranes with some of the properties of the plasma membrane. These will be described in a later chapter. Traube's membranes are easily permeable to acids and bases; halides and nitrates of alkalis, many amines, urea and H_2O_2. They are poorly permeable to sulphates, phosphates, carbonates and all salts of alkaline earths and heavy metals, salts of many organic acids, four substituted derivatives of NH_3, glycerine, basic and many acid dyes, but impermeable to sugars.

Overton (1899) studied the permeability of plant cells by means of the plasmolytic method, and arranged substances in groups according to their penetration power as follows:

1) Those substances which penetrate slowest are neutral salts of strong acids and alkalis or alkaline earths, many neutral salts of organic acids or bases, especially acids with short carbon chains, amino acids, and hexoses.

2) Erythrite (4-hydric alcohol).

3) Glycerine (trihydric alcohol), urea and thio-urea.

4) Dihydric alcohols and amides of monovalent acids.

5) Those which penetrate fastest are monohydric alcohols, aldehydes, ketones, aldoximes, ketoximes, and mono, di and tri halogen hydrocarbons, nitriles, nitroalkyles, neutral esters of inorganic and organic acids, and many organic acids and bases, and ammonia, CO_2 and the elementary gases. Also free alkaloid bases and some basic dyes.

Increasing numbers of hydroxyl groups decrease the penetrating power, and the same effect results from increasing the number of NH_2 groups. Halogen substitution increases the penetrating power. The alkyl or acetyl substitution of the OH or NH_2 groups increases the penetrating power.

As a rule the basic dyes penetrate easily, and the sulphonic acid dyes less easily, but there are exceptions. If the cell is placed in a dilute solution of the dye the minute size of the cell makes it hard to distinguish whether the dye has penetrated or not, since the microscope does not concentrate the color. If the cell is placed in a concentrated solution of the dye and then returned to a colorless medium for observation, the dye that has penetrated may come out again before it can be observed. Many of the basic dyes are precipitated by tannin in the cells so that they may be observed, or they may stain granules or gels. Some dyes that do not at first become more concentrated in the cells than in the medium, become more and more coarsely colloidal within the cells, which constantly absorb more dye in true solution, until they are stained sufficiently to be observed. It is best, in this case, to place the cut stem of the plant in the dye and allow it to remain several days before observation.

Overton's work has not all been confirmed. Janse states that KNO_3 penetrates Spirogyra rapidly. Osterhout (1909) claims that calcium and other salts penetrate plant cells at a fair rate. He supposes that Overton may have failed to observe his experiments continuously.

It is well known that plants concentrate some of the salts of the water that bathes their roots. This is not entirely due to the evaporation of the water from the leaves, since the same is true of water plants. The ash of the kelp, Nereocystis, of the Pacific coast may contain 67.5 per cent KCl. The KCl in the kelp juice is 0.38 n and in sea water 0.01 n. The ash of Iridaea edulis contains 23.42 per cent K=45 per cent KCl. According ot Stoklasa, however, the ash of Azobacter is almost pure K salts (laragely phosphates). The mechanism by which the potassium is selectively absorbed is unknown. According to Meigs and Atwood (1916) if muscle is placed in pure KCl solution it absorbs the KCl faster than the water.

It is certainly true that some neutral salts penetrate some plant cells with exceeding slowness. It was observed by chemical analysis that several grams of yeast absorbed not more than two milligrams of $MgCl_2$ from a molecular solution in five hours (McClendon, 1912 d). Furthermore, Paine (1912 a) used chemical methods and found that yeast absorbs very little if any from more dilute solutions of neutral mineral salts, whereas it absorbs alcohol rapidly. The partitiȯn of ethyl alcohol between yeast and water is 0.85 to 1.

The permeability to injurious substances will be considered under changes in permeability.

The permeability of animal cells is very similar to that of plants. Overton (1902 a) studied the permeability of frog's muscle, concluding that it is essentially the same as that of plants. The relation of muscle to salts is of special interest. Muscle and blood cells store potassium and it is supposed that if they are permeable to salts the potassium will come out and sodium go in, until equilibrium with the blood plasma is reached. Overton claims that neutral salts that do not injure muscle do not penetrate it to any extent. When placed in an isotonic solution of a non-toxic potassium salt, such as the tartrate, the weight of the muscle did not change in fifty hours, but when placed in a toxic isotonic solution, such as KCl, the muscle soon began to swell and died, so that it did not regain its original weight if returned to Ringer's solution. Meigs and Atwood (1916) and Siebeck (1913) claim that muscle is permeable to K, but Urano (1908 c) and Fahr (1909) were unable to wash out the potassium from muscle with isotonic sugar solution.

The permeability of erythrocytes seems to be similar to that of plant cells according to the researches of Grijns (1896), Hedin (1898) and others. Urea, however, seems to form an exceptional solute to which the corpuscles are permeable. Hamburger (1916 b) claims that the erythrocytes are permeable to anions and to $Na^{\cdot}$ and $K^{\cdot}$.

According to Rona and Michaelis (1909 b) the erythrocytes of dogs and men are permeable to dextrose, judging from the partition of dextrose between corpuscles and plasma (which was not constant, however). On the contrary Kozawa (1914 b) found the erythrocytes of swine, goat, sheep, horse, ox, cat, rabbit and guinea pig very poorly permeable to pentoses, pentites, hexoses, hexites, methylated sugar, disaccharides, amino acids and salts of organic and inorganic acids, and the erythrocytes of men, apes and dogs, the same to all except monosaccharides.

The permeability of other animal cells seems to be similar to that of erythrocytes except in the case of certain cells of the kidney, gut, connective tissue and smooth muscle, which are more permeable. According to Meigs (1912 c) smooth muscle cells have no plasma membranes. The composition of other cells in regard to salts is similar to that of the erythrocytes, the Na content of all the cells is less than that of the blood plasma. Whereas the K content of the blood plasma is about .026 per cent, that of the heart is .161, liver .1718, kidney .1643, spleen .1691, brain .245, lung .0836, muscle .3, and that of erythrocytes .5 per cent.

The convoluted tubule cells of the kidney are permeable to many dyes that do not stain other cells. According to Siebeck (1912) they are more permeable to KCl than to other salts.

As is well known, phagocytes can take up solid objects, and might include some fluid containing dissolved substances along with them. The "pyrrol" cells of the connective tissue are considered by Evans and Schuleman (1914) to take up colloidal dyes "phagocytically" but just what is meant is not clear. The Kupfer cells of the liver also take up these dyes, and J. Voigt (1914) claims that they take up colloidal silver. The reticulum cells of the spleen and interstitial cells of the testicle behave similarly toward these dyes.

The eggs of some marine (Fundulus), migratory (smelt) and fresh water fish (pike, muskalonge) are impermeable to salts and also to water. If Fundulus eggs are taken from the mother or from the sea water in which they are laid, they neither swell nor lose their salts. The fish that develop from these eggs are permeable to water, but there is evidence to indicate that except for the gut and kidneys they are impermeable to salts (Sumner, 1905 and 1907, and G. G. Scott, 1913, a and b).

CHAPTER IX

CHANGES IN PERMEABILITY OF PLANT CELLS

That the permeability of a cell may change has long been known. Nägeli (1855) observed that when plant cells died chlorophyll and other substances diffused out of them. Pfeffer ("Pflanzenphysiologie") explained the movements of plants as due to changes in permeability. It had been shown by Pfeffer and de Vries that the osmotic pressure of many plant cells is very high—when the cells are placed in water, that they do not burst is due to the strength of the cell wall. If water is accessible the plants are firm and elastic as a rubber tube when it is filled with water under pressure. If the water is removed from them by evaporation or by immersion in a concentrated salt solution they wilt and contract. The same is true if the permeability of the cells is increased, as by killing the plant by immersion in hot water or in some toxic solution or gas. But changes in permeability may occur without death of the plant. Such changes follow some external change which we call a stimulus. Plants which respond in this way to stimuli are called sensitive plants. The leaves of Mimosa pudica, and many Leguminoceae and Oxalaceae close after being touched. The stamen hairs of Cynara (French artichoke), Centaurea, Berberis and Helianthemum shorten after an insect walks over them.

Pfeffer observed the shortening of these stamen hairs under the microscope. A fluid exuded from the cells into the intercellular spaces, due to increased permeability of the cells to the osmotic substances which held the water in them. The cell walls had been stretched by an osmotic pressure of about three atmospheres. When this was released the cells decreased in volume and the hair consequently decreased in length. The cells in such cases are not dead because they gradually absorb water and swell (regain their turgor) and the hair lengthens. The osmotic substances that were lost when the permeability increased cannot be used to do the work of restoring the turgor, but new osmotic substances must be produced by the cell. According to van Rysselberghe (1899) some plant cells may increase their osmotic pressure by transforming starch into oxalic acid, but his evidence for this is probably slight.

Pfeffer extended these observations to the pulvinus of the sensitive plant leaf. His observations have been confirmed recently by Lepeschkin (1908), who showed that the osmotic pressure of these cells was decreased when they were stimulated and that not only water, but also dissolved substances, diffused out of them. Waller (1904) showed that increased electrical conductivity followed stimulation.

The cells of the sensitive plants are not alone in their changes in permeability. Lepeschkin (1908) and Tröndle (1910) found that light increases the permeability of plant cells to salts and in a lesser degree to dextrose. Fluri (1909) observed that the salts of aluminum, yttrium and lanthanum increased the permeability of plant cells to salts and to sugars. After washing out the reagent the cells returned to their normal condition. If Spirogyra were treated with one of these salts, the tannin diffused out of it so that if it were placed in a solution of chinin, no precipitate of this alkaloid and the tannin was formed inside the cells. Fluri interprets these results differently, however, owing to the fact that the viscosity of the protoplasm is increased so that the chromatophores cannot be precipitated with the centrifuge.

De Vries showed that nitric acid increases the permeability of Tradescantia cells to KNO_3. In experiments with acids it is necessary to distinguish three conditions: First, the cells may be permeable to the acid, as in case of fatty acids. Second, the permeability of the cell may be reversibly changed by the acid, as in case of mild treatment with fatty acids. Third, the cell may be killed by the acid and its permeability irreversibly increased, as in case of the mineral acids. In this experiment of de Vries the cells were probably killed by the acid.

It was shown by Pfeffer that the anthocyan in plant cells may be used as an indicator to show the penetration of acid. The penetration of acid into the cells of the red beet, red cabbage and red nectar glands of Vicia faba has been observed (Mc-

Clendon, 1912 d). The mineral acids penetrate, but the fatty acids more quickly. It is probable that both of these acids increase the permeability.

The same caution should be observed in considering the permeability of cells to bases. E. N. Harvey (1911) stained Elodea leaves with the indicator, neutral red, and studied the penetration of bases. Ammonia and the amines penetrated more quickly than the alkalis. All of these bases probably increased the permeability.

In the experiments of Czapek (1910) the cells were evidently killed and the permeability irreversibly increased. Czapek determined the exit of tannin from Echeveria leaves by the failure of alkaloids to cause a precipitate within the cells. Monovalent alcohols and ketones, ether, urethane, di and tri acetin, sodium oleate, oleic acid, lecithin and cholesterin increased the permeability of the cells to tannin, when the surface tension of their solutions in water sank to about 0.68. Mineral acids increased the permeability when the concentration just exceeded 1/6400 normal, the same concentration at which Kahlenberg and True (1896) had found the growth of Lupinus to be stopped. Their action is evidently due to the H ions.

The experiments of Osterhout (1911) are especially valuable because he was careful that the cells were not killed. He found that the permeability of Spirogyra is increased by pure solutions of NaCl and that this action is inhibited by the addition of $CaCl_2$. A solution of NaCl may be hypertonic and yet fail to plasmolyze the cells because it penetrates, but if a little $CaCl_2$ solution be added, plasmolysis occurs even though the osmotic pressure of the salt solution is slightly reduced.

The electric conductivity method was used to determine permeability of animal cells by several investigators (G. N. Stewart, 1897, McClendon, 1910 c). Osterhout (1912 a) extended this method to seaweed, finding that the conductivity of leaves of Laminaria (kelp) is increased by immersing them in NaCl, and decreased by immersing them in $CaCl_2$ solutions in a reversible manner. He claims that the size of the cells was not changed by the solutions and since the conductivity of the solutions was the same as that of sea water, the result is not due to a change in the conduction in the intercellular substance. He concludes, therefore, that Na increases, and Ca decreases, the permeability of the cells to ions. The intercellular substance in this and many other seaweeds is of large volume in comparison to the volume of the cells. The ash of Laminaria digitata is 22.4 per cent K and 24 per cent Na. It seems certain that this amount of Na might be contained in the sea water in the intercellular substance and hence the cells be free from it. Evidently the cells store K but the mechanism of this storage is unknown.

Osterhout (1915 b) observed that salts of La, Ce, Y, Fe, Al and Th first decrease and then increase the conductivity of Laminaria.

The experiments of Osterhout suggest an explanation of the function of salts in relation to plants. It was shown by Nägeli, Pasteur, Raulin (1870) and others that plants require certain salts that apparently do not enter into the composition of carbohydrates, proteins, or fats. Loew showed that these salts are protective rather than nutritive. Thus, he found that either Ca or Mg in excess is toxic, but that these two poisons antagonize the toxicity of one another. The experiments of Osterhout indicate that they preserve the normal permeability of the plant cells by their contact with the plasma membrane. To speak more generally, we may say that their presence outside and inside the cells affects the aggregation state of the colloids, and that they affect the plasma membrane, which is probably colloidal.

Potassium is stored in many plants and probably all of it is needed, while on the contrary, the calcium that is stored in plants in the form of insoluble salts seems to be a waste product. Sodium is needed by some marine plants (Osterhout, 1912 b). The carbonates, nitrates, phosphates and sulphates are decomposed by the plant in obtaining the carbon, nitrogen, phosphorus and sulphur for the manufacture of proteids and are in this capacity nutritive, but the chlorides are protective. The carbonates and phosphates also serve to preserve the hydrogen ion concentration. Before the leaves fall in the autumn the potassium is absorbed back into the tree, but the calcium is allowed to remain in the leaf.

CHAPTER X

NEGATIVE OSMOSE AND THE POLARIZATION OF MEMBRANES IN RELATION TO THE BIO-ELECTRIC PHENOMENA, STIMULATION, ABSORPTION AND SECRETION

Owing to the wide hiatus yet remaining in our knowledge of this subject, we need especially to guard against definitive statements. It seems best to attempt to explain the unknown in terms of the known, or at least to admit the possibility that the unknown is similar to the known and the invisible of the same structure as the visible.

Of the various conceptions of osmotic membranes, two seem to stand out prominently. The first is that the membrane is a molecule sieve. The fact that the osmotic properties of porcelain membranes increase as the pores become finer, tends to support this view. On this hypothesis, the electric phenomena associated with membranes become capillary electric phenomena. The second conception is that an osmotic membrane is a separate phase that will dissolve some of the constituents of the fluids bathing it. The electric phenomena associated with such membranes are called phase boundary forces by Nernst and Riesenfeld. Since there must be spaces between the molecules of the membrane, this phase becomes a sieve and the difference between the two conceptions seems to be more quantitative than qualitative. It may be asserted that from the first viewpoint we are concerned with the size of pores and molecules, and with adsorption and contact electricity, whereas from the second viewpoint we are concerned with solution, partition coefficient, association and dissociation. But owing to the state of our knowledge of the forces concerned in these phenomena, it is difficult to compare them. Presumably all forces are electric. The dielectric constant of the phase membrane determines its ionizing power and hence its permeability to ions, but the dielectric constant of the sieve membrane determines its electric charge, and hence its permeability to ions.

If we try to classify membranes according to their permeability, the boundary between sieves and phase membranes seems to break down. Bigelow and Bartell (1909) showed that the permeability and osmotic properties of porcelain membranes depend on the size of the pores. Whereas the pores in these membranes can be seen, those in gelatine or collodion membranes are invisible. Bechhold (1907) showed, however, that the permeability of collodion or gelatine gels varies directly with the water content, and with high water content large particles could pass through them by filtration. Thus it seems that these emulsoid gels have pores, a fact that may be detected in suspensoid gels with the ultramicroscope. Rubber is also a colloid, at least under certain conditions, although a rubber membrane has been regarded as a continuous phase. Prof. Ira H. Derby tells me that the rate of flow of liquids into a rubber membrane is mathematically the same as the rate of flow into capillary spaces existing in small spherical aggregates, indicating that the liquid passes through capillary spaces in the rubber. This is not only true of native rubber, in which traces of the emulsion structure might supposedly be left, but also of dental rubber, in which the spaces between the original droplets have probably been obliterated by the process of manufacture.

Negative Osmose

It has long been known that absorption by the gut and secretion by the kidney may take place against osmotic pressure and concentration gradient, and many attempts have been made to rob these phenomena of their peculiarly "vital" attributes. Hamburger (1908) claims that membranes of different composition on the two faces show irreciprocal permeability to water and solutes.

These experiments by Hamburger (which have apparently been discontinued) may be concerned with the phenomenon of negative osmose observed by Dutrochet, Graham, Girard, Bernstein and Bartell. According to Flusin (1908 a and b) the negative osmose is due to the fact that the swelling of the membrane is greater on one side than on the other.

The experiments of Bartell (1914) are more illuminating, since he used porcelain membranes that could be studied in a more exact manner. He had already observed that porcelain with large pores shows no osmotic effects and that the semipermeability varies inversely with the size of the pores, seemingly directly with the molecular weight of the solute. The membranes showing negative osmose are very leaky membranes with pore diameters of about 0.2 microns. No osmotic effects were observed with $MgCl_2$ solutions if the pore diameters exceeded 0.4 microns, negative osmose occurring with pore diameters from 0.4 to 0.1 microns and positive osmose with pores less than 0.1 micron in diameter. The fact that the same membrane showed positive osmose with KCl and negative osmose with LiCl indicated a relation of ions to osmose, which will be considered in the next section.

Electric Polarization of Membranes and Its Relation to Osmose

The electromotive force (emf) of a concentration cell is due to the diffusion of ions, but since ions of the same kind diffuse in all directions in water, at equal rates, one or two other conditions must also be present. There must either be a membrane that alters the diffusion rate in one direction or there must be an unequal distribution of other ions. Although the molecules of one non-electrolyte may not affect the diffusion of another, ions exert forces on one another through their electric charges. Take, for example, the forced diffusion of ions in a potential gradient. If an electric current is passed through a dilute solution of HCl, the H ions will carry most of the current because they move nearly five times as fast as the Cl ions. Now add KCl until its concentration is twenty-five times as great as the HCl. The K ions move with the same speed as the Cl ions, hence they would carry only five times as much current as the H ions although twenty-five times as numerous. In a pure HCl solution the H ions carry $5/6$ of the current, whereas in the mixture they carry less than 0.1, moving less than 0.1 as fast as in the pure solution. In this way the addition of KCl reduces the diffusion potential that would be produced at the boundary between solutions of HCl of different concentrations, the more KCl present at the boundary, the greater the reduction. To take a simple case, consider the following concentration cell:

Calomel electrode|.1 normal KCl|.1 n HCl|n HCl|n KCl|cal. electrode.

The diffusion potential between the two acid solutions may be calculated from Nernst's formula (in millivolts at 25°):

$$\text{emf} = \frac{u-v}{u+v}\, 59 \log \frac{c_2}{c_1} = \frac{318-65}{318+65}\, 59 \log 10 = +39 \text{ mv on}$$

the left. The two diffusion potentials between HCl and KCl may be calculated by Planck's formula: $\text{emf} = 59 \log \frac{u_1+v_2}{u_2+v_1}$ and

for the first one is $59 \log \frac{318+65}{65+65} = 59 \log 3.2 = +29$ mv on the left, and is neutralized by the second which is +29 mv on the right. Hence the total emf is +39 mv on the left, but would be less if the two KCl solutions were of the same concentration. In this concentration cell, the emf is due to the difference in speeds of $H^{\cdot}$ and Cl'. If we increase this difference by interposing between the two HCl solutions a membrane permeable to $H^{\cdot}$ but impermeable to Cl' the emf will be increased to 59 mv, or if the membrane is permeable to Cl' and impermeable to $H^{\cdot}$ the emf will be reversed in direction but still be 59 mv. In studying the effect of a membrane on the relative speeds of the two ions of an electrolyte, the use of KCl is most convenient wherever possible, since the two ions move at the same rate, and danger of unbalanced diffusion potentials at the two ends of the series is eliminated.

The effect of porcelain membranes on the emf of concentration cells was studied by Bartell and Hocker (1916). Bartell had observed that negative osmose only occurred if the speed of the anion in free diffusion were greater than the cation, and Hocker found that in negative osmose the speed of the anion through the membrane is greater than the cation. Since the dilute side receives the charge of the diffusing ions, the dilute side is negative in negative osmose and positive in positive osmosis. The porcelain itself is charged negatively except in relatively more concentrated solutions of acids, or the salts of certain polyvalent or heavy or rare metals, which neutralize or reverse the charge and consequently alter the osmose. Apparently zinc ions cannot reverse the charge of the porcelain. The author observed that if a concentrated solution of zinc sulphate were placed on each side of a porcelain membrane (kindly loaned by Dr. Bartell) and an electric current passed, water was drawn through the membrane to the cathode by electroendosmose. This showed that the water was charged positively, and the water on passing through capillary spaces always takes the opposite charge to the charge of the walls.

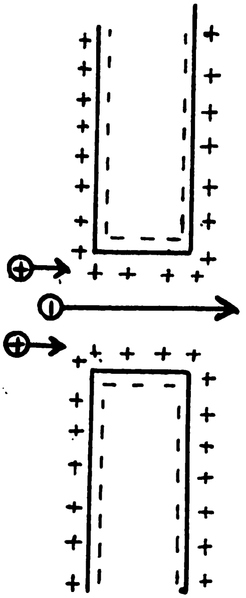


FIG. 27. Scheme showing the mode of diffusion of anions through a large pore in a negatively charged membrane, thus causing negative osmose by electroendosmose.

Bartell and Hocker explain negative osmose as electroendosmose. Since the dilute side becomes charged negatively, it pulls the positively charged water through the membrane, causing the flow to be toward the dilute side.

In Fig. 27 is represented a negatively charged membrane with a large pore. If the pore were small a negative ion could not enter it since it would be repelled by the negative charge of the walls. If cations pass through small pores they tend to make the dilute side positive and hence favor positive osmosis by electroendosmose, hence a membrane containing only such pores would show only positive osmosis. Anions can diffuse through the large pore, however, and since they diffuse faster than cations in case of those salts effecting negative osmose, they give the dilute side a negative charge. The water, therefore, that is charged positively (near the walls of the large pore) is pulled through toward the dilute side. We have, then, in favor of negative osmose, the electroendosmose and the water carried by the electrolyte that diffuses toward the dilute side; whereas, against negative osmose, we have the osmotic pressure of the more concentrated solution exerted rather ineffectively against a leaky membrane. It may be added that the explanation given by Freundlich (1916) seems at least unnecessary.

It was further observed by Hocker that not only the magnitude but also the direction of osmose is affected by changes in concentration of the electrolyte. Negative osmose did not occur with solutions more dilute than n/50. Perhaps at this dilution the size of the negative ions is so increased by hydration as to prevent them from passing through the pores. It would be interesting to know whether more dilute solutions show negative osmose with membranes having slightly larger pores.

The experiments of Bethe (1911) and Bethe and Toropoff (1914) may be interpreted as indicating that the charge in the water half of the electric double layer over the porcelain is due, in part at least, to H ions. They found that when an electric current of more than one volt is passed from non-polarizable electrodes through a Na_2SO_4 solution, divided by a diaphragm of gelatine, jelly, collodion, agar-agar, albumin, bladder, gold beaters skin parchment, carbon or clay, the anode side becomes alkaline and the cathode side acid, whereas water passes toward the cathode. Thus water and hydrogen ions pass in the same direction. The water therefore that is charged positively contains an excess of H ions, which must give it at least part of its positive charge. The side of the membrane toward the anode is alkaline on account of the removal of hydrogen ions. It is impossible for the massing of anions against a non-metallic membrane to cause an acid reaction, since there is no means of robbing them of their charges.

Bethe and Toropoff found that polyvalent cations tend to reverse the charge of the membrane and, hence, the direction of electroendosmose and disturbance of neutrality, whereas polyvalent anions tend to maintain it. The effectiveness of anions in causing cathodic acidity is as follows: citrate$'''>$ phosphate$''>$ oxalate$''>$ $SO_4''>$ $I'>$ $Br'>$ $Cl'>$ NO_3'. The effectiveness of the cations in reversing the charge of the membrane and disturbance of neutrality is as follows: $Co(NH_3)_6^{\cdot\cdot\cdot}>$ $La^{\cdot\cdot\cdot}>$ $Ca^{\cdot\cdot}>$ $Ba^{\cdot\cdot}>$ $Mg^{\cdot\cdot}>$ $Na^{\cdot}>$ $Cs^{\cdot}>$ $K^{\cdot}>$ $Li^{\cdot}>$ $NH_4^{\cdot}$.

The experiments of Bethe on living cells (1916) are, however, open to another interpretation. It was shown (McClendon, 1914 d) that the anthocyan (or other pigment) in many plant cells is amphoteric. In acid it is red and goes to the cathode, whereas in alkali it is blue or green and goes to the anode, if an electric current is passed through the solution. These changes may be observed in the living cells, which are penetrated easily by acetic acid or ammonia but are easily injured by the reagent or current so that the pigment passes out of them. In successful experiments, all of the pigment is massed in the cathode ends of red cells and in the anode ends of blue cells. When the plant is fresh the pigment is usually reddish, but contains a small proportion of blue, and sometimes violet cells may be found. If an electric current is carefully passed through a violet or red-violet cell under the microscope, the red portion of the pigment will mass in the cathode end of the cell, and the blue portion in the anode end. This phenomenon may have led Bethe to suppose that the passage of the current through the membrane disturbed the reaction. His experiments on cells stained with neutral red are not, however, open to this objection. It should be noted that the anode end of the cell is the cathode side of the membrane against which the pigment is massed.

It might be supposed that the colloidal membranes used by Bethe and Toropoff are so different from the porcelain used by Bartell that the results may not be compared. Bethe and Toropoff found, however, that membranes of clay or carbon behaved in the same way as gelatine membranes, in the disturbance of neutrality on the passage of an electric current through them. We may, therefore, safely use the foregoing findings in explaining the results obtained with various membranes. Flusin

(1908) observed that a solution of tartaric acid passes through pig's bladder into pure water (negative osmose). Evidently the acid reversed the charge on the membrane, making it positive, the greater speed of the positive ion causing the negative osmose. Although Flusin adopts another explanation for anomalous osmose, Girard (1908-13) shows conclusively that electroendosmose is at least in part the correct explanation. If the solutions on the two sides of the membrane are isotonic the driving force of osmose is the diffusion potential causing electroendosmose. In the following table the first column gives the solutes used in the isotonic solutions, the second the charge of the membrane, the third column the direction of the emf (positive current), the fourth the direction, and the fifth the relative magnitude of the osmose.

Isotonic solutions	membrane potential	diffusion emf	direction of osmose	magnitude of osmose
Sucrose Tartaric ac.	+	↑	↑	33
Sucrose $Pb(NO_3)_2$	+	↓	↓	10
Sucrose K_2CO_3	—	↑	↓	30
Oxalic ac. Na_2SO_4	+	↓	↓	20

With a membrane separating isotonic solutions of NaCl and KCl there was no emf and no osmose. The same was true of KCl and Na_2SO_4. Although these experiments of Girard antedate those of Bartell and Hocker, the latter have been described first on account of the simple character of the membranes and the remarkable relation of the diameter of the pores to their properties. Although it may not have been shown that all anomalies in osmose are due to electroendosmose, it seems unnecessary at present to accept the hypothesis of Flusin that osmose takes place through the membrane from the side of least swelling (smaller pores) to the side of greatest swelling (largest pores). At any rate, the size of the pores in *porcelain* membranes is not affected by the character of the solute (Hofmeister series).

Bernstein ("Elektrobiologie," p. 162) supposes electroendosmose to be the explanation of his observations on negative osmose through copper ferrocyanide membranes, although he records no observations on the electric charge of the membranes or the emf. On filling the osmometer with K_4FeCy_6 solution and immersing it in $CuSO_4$ solution of slightly higher osmotic pressure (calculated from the freezing point) he observed a rise in the manometer, in some cases overflowing at 34 cm. In order to eliminate any possibility of error from the unequal temperature coefficients for the osmotic pressures of the two solutions, he repeated the experiments at 0°, and yet the manometer overflowed. The emf of these membranes as measured by Brünings are given below.

Comparison of the Polarization of Various Types of Membranes

The behavior, mentioned above, of the copper ferrocyanide membrane may seem surprising, because, of all dead membranes that have been investigated, it has proved to be the most truly semipermeable in osmotic experiments. Brünings (1907) using isotonic solutions of $CuSO_4$ and K_4FeCy_6 observed that the $CuSO_4$ side is electropositive. He supposed the emf to be due to the permeability of the membrane to K ions, and its being impermeable to the other ions. If this were the case, the emf should be proportional to the logarithm of the concentration of K ions (which is not true, as the emf remained very near 100 mv with large variations of K ion concentration). It has been shown by H. N. Morse (1914) that these membranes rapidly deteriorate when in contact with other ions than those from which they are formed, and for this reason it may be impossible to decide this question. When we remember, however, that Morse observed that the membrane may be greatly thickened by the passage of an electric current, it becomes clear that the membrane cannot be absolutely impermeable to copper and ferrocyanide ions until its electric conductivity becomes practically zero. Evidently, during the formation of the membrane (without the aid of the current) negative charges are removed from the ferrocyanide solution and positive charges are removed from the copper solution, which would produce an emf opposite to that found by Brünings if the membrane is impermeable to all ions except copper and ferrocyanide ions.

According to Beutner (1913, b) copper ferrocyanide membranes are permeable only to the cations of many salts. In order to prevent deterioration of the membrane he set up this series: Calomel electrode$|K_4FeCy_6|CuSO_4+x$ mol. salt|calomel electrode. With n/40 NaCl in the $CuSO_4$ solution the emf was 29 millivolts, and with n/400 NaCl in the $CuSO_4$ solution the emf was 80 mv, or a difference of 51 mv produced by diluting the salt ten times, whereas the difference calculated by Nernst's formula is 58 mv.

Taking Beutner's results as evidence that these membranes are permeable only to the cations of the salts of alkali metals, we may explain the negative osmose observed by Bernstein on the assumption that the membrane is negative, hence the water positive, the diffusion of the K ions leaving the ferrocyanide solution negative.

One of the clearest demonstrations of the relation of permeability to membrane polarization was shown by Bayliss (1911). He separated two solutions of the sodium salt, congo red, by a membrane of parchment paper. The Na ions can easily pass through the membrane but the congo red anions cannot pass through a dense grade of the paper. Therefore the dilute side is electropositive. Bayliss found the emf, when measured immediately after the membrane was wet with the solutions, to approximate that calculated by Nernst's formula, but to fall gradually. He explained the fall by assuming that the osmotic pressure of the concentrated solution draws water through the membrane, thus forming a dilute layer next to the membrane and lowering the difference in concentration between the solutions in immediate contact with the two faces of the membrane.

The author found that even with KCl solutions, parchment paper gives an emf, but the potential difference falls very rapidly, so that it is impossible to measure the initial potential with the potentiometer method used. The fall in potential is not entirely due to dilution of the more concentrated solution, since after removing and partially drying the membrane and renewing the solutions, the original potential is not obtained. Since the emf becomes zero after a comparatively short time, the fall is probably due, at least in part, to swelling of the membrane and consequent enlargement of the pores. In one experiment an emf of

40 mv was observed. With a wooden membrane and KCl the emf was 7 mv, and the dilute side positive.

The emf produced by porcelain membranes in Hocker's experiments was very small, due to the fact that he waited twenty-four hours for equilibrium to occur before making the reading. He found that time to be required owing to the thickness of the porcelain. Very irregular results were obtained if the reading were taken soon after filling the apparatus. The author observed that the bowl of a clay pipe showed negative osmose and was thin enough to come to an equilibrium more quickly, provided the previous solution had been thoroughly soaked out of it. Brünings (op. cit.) found Nernst's formula to be approximated with a burned clay membrane separating n/100 and n/1000 NaCl solutions, the observed emf being 50 mv and the calculated 59 mv. Brünings obtained rapid equilibrium by soaking the membrane in the more concentrated solution, in one case boiling it in this solution sixteen days with a reflux condenser. Brünings tried membranes of burned clay, wood, bone, carbon and marble with NaCl and KCl, and in every case the dilute side was positive. Since the Cl ion is faster than the Na or K ion these membranes must be more permeable to cations than to anions. Probably this is due to repulsion of the anions by the negative charge of the membrane.

The effect of the electric charge of the membrane on its relative permeability to anions and cations is illustrated by an experiment of Mines' (1911 b). In the following concentration cell the emf is zero:

$$\text{Zn, ZnSO}_4 \mid \text{n/8 NaCl} \mid \text{n/80 NaCl} \mid \text{n/8 NaCl} \mid \text{ZnSO}_4\text{, Zn.}$$

If, however, a gelatine membrane is placed between two of the NaCl solutions the dilute side becomes positive with an emf of about 60 mv. The gelatine, therefore, is more permeable to cations than to anions, and it is well known that natural gelatine is electronegative. Gelatine is made positive by the ions of polyvalent metals, and hence Mines treated the gelatine with a salt of Gadolinium to test its effect on the emf. The same gelatine membrane used above, after treatment with $GdCl_3$ and being returned to the concentration cell, caused an emf of 8 mv, but the dilute side was negative, showing that the membrane had become more permeable to anions or less permeable to cations —perhaps both.

Selective permeability of membranes to ions has often been noticed in electrical transference experiments. Hittorff separated the solution between the electrodes into segments by means of ox gut membranes, in order to prevent backward diffusion or convection currents. He supposed the membrane did not affect the results (transference numbers), but it is now known that the effect is small in some cases, very large in others. Bein (1899) found that earthenware membranes were the safest to use. Fish bladder membranes caused but small errors with neutral salts of alkali metals, but large errors with $CuSO_4$ solutions. The SO_4 ions passed the membrane, but the Cu ions combined with the substance of the membrane, and hence were held back.

The action of gelatine membranes in holding back H ions in a similar manner has been noticed by Girard and by Cybulski (1903) and Cybulski and Dunin-Borokowski (1909).

Where the membrane clearly forms a separate phase the electric phenomena have been called by Nernst and Riesenfeld (1902) phase boundary forces. If the speed of the ions in two phases is different, the addition of the electrolyte to either phase will cause a difference of potential at the phase boundary, which is different from that which occurs in a similar concentration gradient in either phase alone. The limiting case is that in which the speed of either ion becomes zero (in the membrane). Such is the case with a metallic membrane, which may be considered impermeable to any ion except the cation of the same metal. Although the atom and its electron part company in passing through the membrane, and the ion as such does not pass through, the effect is the same, since an ion is given off from one side at the instant that an identical ion is deposited on the opposite side of the membrane. It seems proper, therefore, to speak of the permeability of a metallic membrane to an ion, when the mechanism of the passage of the ion or its components through the membrane is of no significance to the point of discussion. When an electrolyte dissociates into two ions to only one of which the membrane is permeable, the emf may be calculated by Nernst's formula for electrode potentials. The emf is 59 mv at 25°, when this ion is monovalent and is ten times as concentrated on one side of the membrane than on the other.

It is characteristic of most of the separate phase membranes that have so far been studied that they are very poorly permeable to water. Their ionizing power is often very small, and excepting the metallic membranes, they are poor electric conductors. Hence the measurement of the emf with a galvanometer, capillary electrometer, or even a potentiometer, is sometimes impossible. A quadrant electrometer of Lord Kelvin's or Dolezalek's type is usually used.

For the general case of a membrane permeable to both ions of the solute, Nernst's ideal formula in millivolts at 25° is,

$$\text{emf} = 59 \left(\frac{u-v}{u+v} - \frac{u'-v'}{u'+v'}\right) \log \frac{c_1}{c_2}$$

where *u* and *v* are the speeds of cation and anion in water and *u'* and *v'* in the membrane. Though the emf so calculated would be correct if the two aqueous solutions were connected so as to form a closed circuit, the emf could not be measured under such conditions. If calomel electrodes are used, the solutions connecting this concentration chain to the electrodes would have an effect on the total emf as measured.

Examples of membranes permeable to only one of the two ions of an electrolyte are given by Cremer (1906) and Haber and Klemensiewicz (1909). Membranes of ice, benzol, toluol, metaxylol, nitrophenol, and thin, water soaked glass, acted as though they were permeable only to H ions. When the H ion concentration on one side of the membrane was ten times as great as on the other side, the average emf with soft glass was 52 mv and with hard glass 59 mv, whereas that calculated from Nernst's formula is 58 mv at 20°. The emf with the other membranes was almost as great. Haber and Klemensiewicz used almost pure solutions of acids and bases (without buffers) and, in their tables, did not correct for dissociation. Furthermore, they made no accurate measurements near the neutral point. Since the reaction of all living cells or fluids bathing them is near the neutral point, it seemed worth while to make such determinations, in order to ascertain whether such membranes might account for the bioelectric phenomena. Prof. A. F. Kovarik kindly took the readings with an improved Dolezalek's electrometer. Benzol was used as a membrane. Its conductivity is so low that the concentration cell had to be enclosed in a metal cage before constant results were obtained. The H ion solutions were PH = 6 and 7 made with the aid of Sörensen's phosphate mixtures and tested with the hydrogen electrode. The emf was 31 mv, whereas the theoretical is 59 mv. This is not, however, a greater discrepancy than in some of Haber and Klemensiewicz's experiments with benzol, the potential difference between n/10 and n/100 HCl being only 18 mv. The emf of living muscle as measured, is not usually greater than 31 mv, and since the PH of the blood is about 7.5 the reaction of the muscle contents need only be PH = 6.5.

Whereas, in the above membranes, their chemical nature seemed unimportant, ice, glass and carbocyclic compounds behaving in the same manner, the permeability of certain other membranes depends, according to Beutner (1913 d), on a chemical reaction. Beutner used one series of membranes permeable only to cations and another only to anions. An example of the first class is salicylic aldehyde, that on oxidation formed traces of salicylic acid. If such a membrane separates solutions of the same salt of different concentrations, the dilute side becomes negative. If both solutions are very dilute, Nernst's formula may be used to calculate the emf approximately. According to Beutner the cations pass through the membrane by being transformed into salicylates, which are soluble in salicylic aldehyde. Beutner's experiments were repeated with n/100 and n/1000 KCl on the two sides of the membrane, and an emf = 36 mv obtained on Prof. Kovarik's electrometer.

The conductivity of the salicylic membrane is too low to use the potentiometer if the ordinary U-tube is used in making the concentration cell, but at least qualitative results were obtained in the following manner: A solution of $CaCl_2$ of greater specific gravity than the aldehyde was placed in the bottom of a beaker and a glass tube introduced into it. A layer of salicylic aldehyde (to which some salicylic acid had been added to increase its conductivity) was floated on top of the $CaCl_2$ solution, and a dilute solution of $CaCl_2$ added as a third story. In this way a membrane of larger surface and less thickness was made. On connecting one calomel electrode with the dilute solution and the other through the glass tube with the concentrated solution, an emf of 25 mv was measured with the potentiometer, the dilute side being positive.

The other type of membrane is an organic base, which, according to Beutner, combines with the anion of the electrolyte to form a salt soluble in the membrane. His experiment was repeated with o-toluidin, in the same manner as with salicylic aldehyde, and obtained 27 mv with the Dolezalek electrometer. In order to use the potentiometer, chloroform was added to the toluidin to make it heavier, and 80 mv obtained, the dilute side being negative.

In order to obtain larger membranes, parchment paper tubes were soaked in salicylic aldehyde, toluidin or anilin. In each case the emf was what would be expected with parchment paper alone, but was maintained for a longer time. For this reason it seems possible that the organic liquid was effective in retarding the wetting of the paper with consequent enlargement of its pores. If this is true it shows a method of finding the initial emf with parchment paper. Although with toluidin alone the dilute side is negative, when it is applied to parchment paper the dilute side is positive. The emf with n/100 and n/1000 KCl was 59 mv, and substituting toluidin, was 52 mv.

According to Loeb and Beutner (1913) a solution of lecithin in m-cresol acts in the same way as salicyl aldehyde when used as a membrane in a concentration cell. But since higher fatty acids could be substituted for lecithin, it seems probable that fatty, or glycerophosphoric acid, formed by the decomposition of lecithin was the active agent.

Bioelectric Phenomena

The electromotive forces produced by living matter have always been a subject of interest, if not amazement. The shock of some electric fish is several hundred volts, and may be felt while the fish is entirely submerged in sea water. On the other hand, the ordinary electric potential differences observed in living matter never reach 0.1 volt. The secret of the electric organ es in the connection of the elements in series. Brünings observed that if one frog's skin is laid on another, the emf is doubled. In the same way he found the emf of two concentration cells with porcelain membranes to be doubled when they were connected in series. The principle is the same as that of the voltaic pile, and only the nature of the membrane is different. A dissection of an electric organ reveals the serial arrangement, and also (in all electric organs related to muscle tissue) that the side of each element on which the nerve enters is electronegative (Pacini). It seems probable that the manner of production of electricity is the same in the electric organ as in muscle or nerve or any sensitive cell. We shall therefore proceed to discuss the general question of the exhibition of electromotive force by the cell.

The electromotive force of a single cell was observed by Hyde (1904). She placed one non-polarizable electrode on the animal pole and one on the vegetative pole of the egg cell of a fish. When the cell began to divide the animal pole became negative.

Owing to the small size of most cells, it is usually more convenient to study these phenomena in tissues in which similar cells are arranged in multiple. The frog's muscle is convenient. It is possible to measure the difference in potential between the inside and outside of the cells by cutting off one end of the muscle and leading off from the cut surface (interior) and the intact surface (exterior). The interior is found to be negative in comparison to the exterior, which causes an electric current to flow through any external conductor that may be present from the intact to the cut surface. This current is often called the current of injury, without any proof that the injury, as such, has anything to do with it, but the expression is convenient.

Wilhelm Ostwald suggested that the muscle is a concentration cell with a membrane, and Bernstein developed the details of this idea. No one doubts that the muscle is a concentration cell, but the opponents of Bernstein's hypothesis doubt that a membrane has anything to do with it, since concentration cells may be made without membranes. But the emf of the current of injury of a fresh muscle is from 40 to 80 mv and no concentration cell without a membrane has been known to give such a high emf unless it contained strong acids or alkalis, which are incompatible with life. There seems to be no valid evidence against, and much for, Bernstein's theory. Furthermore, it is the only theory that will account for all of the facts.

According to Bernstein, the striated muscle fiber or cell is surrounded by a membrane or surface (called the plasma membrane since it is the superficial layer of the protoplasm), which is more permeable to the cation than to the anion of some electrolyte more concentrated in the interior than on the exterior of the muscle cell. The cations passing through the plasma membrane leave the interior negative, and form a positive outer stratum of an electric double layer thus formed at the surface of the cell. Many attempts to determine the electrolyte have been indecisive, but it seems probable that the cation in question is the hydrogen ion, in which case the cell surface must be relatively impermeable to all anions. This assumption has the advantage that it avoids the necessity of difference in osmotic pressure on the two sides of the membrane. A diagram of this idea is shown in Fig. 28, representing a muscle cell with one end cut off to produce the current of injury. The (—) signs are anions that can escape only at the cut end. The current of injury is caused by the escape of the anions at the cut surface. What these anions may be is immaterial, but presumably they include proteids with negative charges and anions of carbonic and lactic acids produced in the muscle fiber.

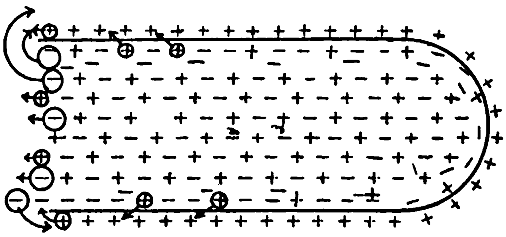


FIG. 28. Scheme showing the plasma membrane of a cell torn open at one end and liberating the excess of anions. The + and — indicate the charge of ions and the arrows the direction of diffusion. Only + ions can come out through the intact plasma membrane and hence there is an excess of — ions on the interior. The tear causes a negative charge to appear at the surface and give rise to the current of injury.

The acidity within the cell necessary to produce such an emf as in muscle need be only that of a molecular solution of CO_2.

The H ion concentration of the blood at 20° is about PH = 7.5 and with such a reaction outside the cell and PH = 6.5 inside the cell, the theoretical current of injury would be 58 millivolts, which is about the average value actually found. With the model membrane given in the first section, the emf did not reach this theoretical value, but it is reached in the average of one series of determinations by Haber and Klemensiewicz.

Not only is the cut surface of a muscle negative, but the region of a muscle that is stimulated becomes negative for a fraction of a second. The current produced by this negativity is called the action current, and since the current of injury is produced by the removal of the resistance to diffusion at the cut surface, the action current is probably due to a momentary increase in permeability at the stimulated surface. If this is true we should be able to increase the electric conductivity of muscle momentarily, by stimulating all of the fibers simultaneously. Since it is not easy to measure conductivity in a few thousandths of a second, even with a string galvanometer on stimulating the nerve, another method of stimulation was adopted (McClendon, 1912 c). The conductivity of a resting muscle was measured by passing an alternating current transversely through it. By putting in resistance this current was made too weak to stimulate the muscle. After the conductivity was determined, the resistance was short circuited and the conductivity determined while the muscle was thrown into tetanus by the alternating current, and found increased from 6 to 28 per cent. It has been objected that the action current of the muscle reinforced the alternating current, but the contrary is true. The muscle is stimulated on the side of the momentary cathode, and the stimulated region, becoming negative, opposes a cathode to a cathode, hence opposes the current. It is also stated that the change in form of the muscle fibres increases the cross section of the interfibrillar spaces. This objection seems to have no theoretical basis. If a vessel is filled with small spheres the cross section of the space between the spheres is the same as though it were filled with large spheres. In my experiments a relatively large muscle was pressed between two platinized platinum discs as electrodes. Between the discs were a large number of cylinders (fibers) and when the musc contracted it was drawn in at the ends and pushed out at the sides, so that the same area lay between the electrodes, but was now composed of a smaller number of larger cylinders. It may be objected that the surface of the contracted fibers is thrown into waves but this does not affect the problem, because such fibers can be resolved into an infinite number of short cylinders of different sizes placed one on another. If a change in the diameter of all of the fibers does not affect the conductivity, a local variation in the diameter of one fiber does not affect it. It seems that the evidence points to an increase in the permeability of the muscle on stimulation.

It was shown by Kunkel (1887) and Burdon-Sanderson (1888) that an action current follows stimulation of the sensitive plant, Dionea, and the same has been found true of a host of plants by A. D. Waller. It seems probable that an action current (blaze current) follows stimulation of any plant except perhaps most marine plants, as indicated by Waller.

This increase in permeability may cause movements (in addition to electric phenomena). Pfeffer ("Physiol. Untersuchungen" 1 and 2, 1873) studied the movements of plants. The stiffness of a plant is due to the pressure or turgor within its cells, and Pfeffer observed that plant movements are caused by local variations of turgor. Each cell is surrounded by a semipermeable plasma membrane. The osmotic pressure within the cell causes the absorption of water, from the capillary spaces between the cells. When the plant is stimulated certain cells lose their semipermeability and the cell sap filters out into the intercellular spaces, causing a local shrinkage of the tissue.

Waller (1904) observed that the conductivity of plants may increase 100 fold (or less) on stimulation, thus giving more evidence for permeability increase. He observed the blaze current to last fifteen minutes in some cases, which makes it easier to study conductivity during the stimulated state.

Whereas the action of the sensitive plant may reach a maximum one second after stimulation, movement does not begin until two and one-half seconds after stimulation. The time required for diffusion together with the mechanical inertia of the parts probably accounts for the delay.

We may sum up the data on the universality of the bioelectric phenomena by saying that the current of injury has been observed in all living tissue where it has been looked for, but that action currents have been observed in the leaves of land and fresh water plants, and in animal nerve (and retina), muscle and glands and electric organs derived from them, as well as in the eggs of fish. These electric phenomena are due to changes in permeability to ions. The excited state (increased permeability) remains but a few thousandths of a second in muscle or nerve but may remain fifteen minutes in plants.

The most wonderful thing about the electrical variation of muscle, nerve or sensitive plant is that it is self propagated. The results of study of electrical stimulation have made the self propagation of the electrical variation a necessary consequence of excitability. It is probably the negative variation of nerve that stimulates another nerve or the motor end plate of the muscle. The medullated nerve fiber is surrounded by an insulating sheath, broken only at the occasional nodes of Ranvier. When a minimal current is sent through a medullated nerve crosswise no stimulation takes place, apparently because of the insulation of the fibers. But when it is sent lengthwise through the nerve it may enter at one node and leave the fiber at another. (The author found non-medullated nerves of Limulus to be as sensitive to a current sent crosswise as to a longitudinal current.) The nerve is stimulated nearest the cathode or apparently where the current leaves the fibers at the nodes nearest the cathode. In other words, the approach of a cathode to the plasma membrane stimulates the nerve fiber. But the stimulated region becomes electronegative and hence a little cathode. It stimulates regions adjacent to it, and the stimulated area spreads like a fire. The reason for this can only be learned when we discover the cause of the increase in permeability of the plasma membrane. H. N. Morse (1914) states that copper ferrocyanide membranes are colloidal and that a crystallization, resulting in increase of the size of the particles, increases the permeability of the membrane. But the size of colloidal particles may be considerably increased without crystallization (by electrical disturbances). Perhaps the plasma membrane is colloidal and the sudden electrical disturbance, due to the approach of a cathode, causes the particles to aggregate into groups. If the plasma membrane is composed of negative colloids like the rest of the cell, the approach of a cathode would tend to lessen the charge on the particles and induce aggregation. The negative charge of cell colloids is illustrated by the author's experiments in passing a constant current through plant (McClendon, 1910, b) and animal (McClendon, 1914, d) cells. Chromosomes, yolk granules, and protoplasm in general pass toward the anode, whereas water passes toward the cathode and forms a blister on some animal cells. If the plasma membrane is composed of the same colloids as the cell interior it is probably negative. It may be objected that these colloids become positive in an acid medium and that this negativity is incompatible with the assumed acidity of cell interiors. But the isoelectric point of most of these colloids is much farther on the acid side than the reaction we have assumed for the interior. Furthermore, the plasma membrane is nearer the blood which is alkaline, and is probably bathed by an alkaline tissue juice. The cell sap in the cells of red flowers has an acid reaction.

The rate of propagation of the excitatory impulse may be 1 mm per second in some plants and 12000 in mammals. It is probably very slow over the bodies of nerve cells and motor end plates, as there is some delay in conduction through these regions. Mayer (1916) found that nerve conduction varies almost as the concentration of electrolytes around the nerve. Lillie (1916 b) supposed nerve conduction to vary with electric conductivity but Mayer has not observed an exact parallel.

Since glands are the seat of an emf, and the skin contains glands, the skin is the seat of an emf which varies with glandular activity. If a constant electric current is passed through the body, the electrical resistance is found to be high. If a galvanometer is interposed in the circuit, the needle may soon come to a constant deflection, but if the subject becomes mentally excited, as on hearing bad news, the galvanometer deflection is suddenly increased. This is called the psychogalvanic reflex, and is used by clinicians and psychologists in the examination of the reactivity of persons. Gildemeister (1915) in a very elaborate and carefully controlled series of experiments, showed that the psychogalvanic reflex is due to increased permeability of the sweat glands, which have been stimulated through sympathetic nerves.

The emf of the frog's skin has been the object of many investigations, but the results so far are for the most part confusing. Schwartz (1915) found that the electrical conductivity of the frog's skin is increased when the nerves to the skin glands are stimulated. It is probable that the emf of the frog's skin is analogous to the action current, or more nearly to the current of injury of muscle, and is due to the permeability of one side of the gland cell and semipermeability of the other, the latter being the seat of the emf. The experiments of Bayliss (1908) tend to support this view. On passing an alternating current through the frog's skin, he observed that it acted to a certain extent as an electric rectifier, allowing a current to pass more easily in one direction than in the other. The same effect was produced by a parchment paper membrane separating a solution of congo red from water. This dye dissociates into Na ions and enormous anions. If the cathode is placed in the water, the current is carried through the membrane by the Na ions, but if the anode is placed in the water, the anions attempt to pass through the membrane to the anode, but are unable to do so.

Perhaps much of the confusion arising from the study of the emf of living tissues is due to the fact that they are made of more than one kind of cell, and that the permeability of the cells may be changed by the experimental procedure. Consider, for example, the excised muscle. If it is bathed on all sides by lymph or blood, any emf observed is probably due to the muscle cells, but if one electrode is connected to it by a more concentrated solution and the other by a dilute solution of electrolytes, the system may form a concentration cell in which the perimysium or muscle sheath is the chief membrane concerned. The fact that porcelain membranes may act in this capacity, shows that relatively permeable membranes must be taken into consideration, even membranes allowing an appreciable amount of filtration. Oker-Blom (1901) studied what he called the contact potential between muscle and certain solutions. This work was extended by Brünings (1907) who attempted to prevent the injurious effect of diluting the salt solutions by the addition of sugar to make them isotonic. He found the contact potential between frog's muscle and sugar solution to be 50 millivolts, with the sugar side positive, and the same result was obtained with dead muscle. Perhaps the perimysium surrounding the muscle, being formed of electronegative colloids, is more permeable to the cations than to the anions of the electrolytes between the muscle fibers, hence the dilute side (in reference to the salt) is positive. When the end of a muscle is dipped in water, the dilute (water) side is positive until the water injures the muscle, when the dilute side becomes negative, the perimysium emf being superseded by the current of injury. Brünings found that when the entire body of a man, a frog or a plant is used as a membrane in a concentration cell, the dilute side is positive.

Loeb and Beutner (1914), who extended Brünings's experiments, oppose all hypotheses on the origin of animal electricity which relate to membrane destruction or selective permeability to ions. They admit, however, that a current of injury is produced by cutting off part of the skin of an apple, and that the same increase in permeability may be produced by merely pressing the skin with the finger. The argument they bring against Bernstein's hypothesis is the similarity between living tissue on the one hand and Beutner's acid membranes or lecithin in guaicol on the other, when used as membranes in certain concentration cells. It seems perhaps more significant that in a previous paper (1912) they interpret their results as showing that the seat of the current of injury is the intact membrane and that it is caused by the increase in permeability or removal of the membrane at the injury.

Having thus sketched the membrane hypothesis, it may be advisable to add additional proofs of its validity. If the membrane theory is correct, change of temperature of the cut end of a muscle should not affect the current of injury since the cut end is not the seat of the emf, but the current of injury should be proportional to the temperature of the intact end. From Nernst's formula emf (in millivolts) $= .198\ T \log \frac{c_1}{c_2}$, where T is the absolute temperature, hence any change in T would cause a corresponding change in the emf. Bernstein ("Elektrobiologie," p. 97) found this to be the case, at least between the temperatures 18°-0°. In case two points on an intact muscle are at different temperatures Nernst's formula becomes emf $= .198\ T_1$

—.198 T_2, which means that a thermocurrent will be produced proportional to the difference in absolute temperature. Bernstein found this to be true also, the warmer end being positive.

Further proof of functional change in permeability of the plasma membrane to electrolytes was found on measuring the conductivity of sea urchin eggs by the Kohlrausch method. The conductivity increases when the eggs are stimulated to begin development (McClendon, 1910 c, e). This was confirmed by Gray (1913, a, b) who observed an increased conductivity in the first fifteen minutes of development. Just what electrolytes were affected by this increase in permeability was not ascertained, but in case of the frog's egg, the author was able to show an increased permeability during the first hours of development to Na, K, Mg, Ca, and Cl (1914 a, 1915 a). The eggs were caused to develop in distilled water by means of electric stimulation, and the salts which diffused out analyzed and compared with those diffusing out of unstimulated controls.

A change in permeability may also accompany pathological changes. It was shown (McClendon, 1913 a, 1914 c) that the eggs of certain fish which are impermeable both to water and to salts, may be made permeable to salts by various toxic substances. Salts diffuse out of these poisoned fish into the distilled water in which they are placed, and (possibly as a result of increased permeability) they develop into monstrosities. An increase in permeability to water was shown by Loeb (1912 b).

If stimulation means increase in permeability, we should expect anesthetics to prevent it, since they prevent response to stimuli. It was observed that the increase in permeability of the eggs of certain fish to electrolytes could be partially inhibited by ether (McClendon, 1914 b, 1915 b).

Since Kite claims that the interior of the cell has the same permeability as the surface, it is probably worth while to emphasize that the effect of temperature change on the emf of the current of injury furnishes evidence that the seat of restricted permeability is the uninjured cell surface. Furthermore, Höber (1910 a, 1912 b, 1913 b) has shown that the electric conductivity of the interior of the cell is greater than that of the whole cell, including the plasma membrane.

Stimulation

Modern theories of electrical stimulation depend on membrane polarization. Nernst accidentally passed through his body a high frequency Tesla current of enormous voltage, such as is used in wireless telegraphy, and observed no stimulation. This led him to formulate his theory of electric stimulation, according to which the current must carry a certain number of coulombs of electricity per unit cross section, before it is reversed, in order to stimulate. In other words, stimulation is due to polarization of membranes, and the polarization must reach a certain minimal value. From this it is evident that the higher the frequency of an alternating current (the shorter the time the current flows continuously in one direction) the greater the amperage required in order to stimulate. Using sine wave alternating currents, this was found to be true within certain limits of frequency, but it appeared impossible to stimulate if frequency reached 100,000 per second. Nernst and Barrat (1904) found that the current required to stimulate = .079 × the square root of the frequency. In order to extend the limits of frequency complicated formulae have been developed to include accessory factors, such as backward diffusion of ions (A. V. Hill, 1910). According to Bethe (1916) the facts fit better the hypothesis that only H ions are concerned in the stimulation. He had found that the passage of a current through a membrane causes the accumulation of H ions on one side and OH ions on the other and supposes that the accumulation of H ions on the outer surface of the plasma membrane causes stimulation. He says that the bare nerve fibers exposed at the cut end of a nerve, or which have grown out of the end on regeneration, can be stimulated by acid that is not hypertonic, and hence does not stimulate osmotically.

The relation of ions to irritability has been much studied, but the problem has not been entirely cleared up. An ion may have one effect at a certain concentration and a different effect at another. K ions are said to depress irritability, but according to Beccari (1915) they increase irritability, when applied within physiological limits to skeletal muscle. It is well known that Na increases and Ca decreases irritability. But irritability is lost in the pure solution of any salt. If muscle is placed in isotonic solutions of the chloride of an alkali metal the irritability disappears least rapidly in NaCl and most rapidly in KCl according to the series:

$$Na < Li < Cs < NH_4 < Rb < K$$

If muscle remains in isotonic cane sugar solution the irritability disappears but if they are returned to a salt solution the irritability may be regained. According to Overton the cationic series is about the same as the one above. According to C. Schwarz the anionic series is: $CNS < I < NO_3 < Br < Cl < CH_3COO < SO_4$, Tartrate $<$ Citrate.

If it is carbonic or sarcolactic acid inside the muscle fiber that causes the polarization of the plasma membrane, this structure must be impermeable to all ions except H ions. The H ions of the sugar may be of the same concentration as the H ions inside the fiber and thus abolish the polarization. If this is the case, however, it is hard to see why the muscle is irritable in a mixture of sugar and neutral salt unless the salt in some unknown way changes the H ion dissociation of the sugar. The sugar solution acts as an insulator and hence makes stronger currents necessary for stimulation of the muscle directly. It might also interfere with the transfer of the negative variation from the nerve to the muscle by reducing the conductivity of the solution immediately over the plasma membrane. I know of no other suggestions that might explain the power of a cane sugar solution in reducing the irritability of muscle.

In this connection it may be of interest that the author found it necessary to make the medium more acid than $PH = 6.5$ in order to stop the pulsations of medusae or the heart of the Conch. The action of ions on these pulsations was studied with a view to determining the probability of the hypothesis of the antagonistic action of ions in isotonic solutions. OH', $Na^{\cdot}$ and $K^{\cdot}$ stop the conch heart in systole, whereas $H^{\cdot}$, $Mg^{\cdot\cdot}$ and $Ca^{\cdot\cdot}$ stop it in diastole. The first group tend to quicken and the second to retard the rate. As observed by Ringer, Mayer and others, however, $Ca^{\cdot\cdot}$ in the presence of other ions may tend to increase the systolic phase of pulsations. We cannot pick out 9 pairs of perfectly antagonistic ions, but the antagonism between $Na^{\cdot}$ and $Mg^{\cdot\cdot}$ is more perfect and between OH' and $Ca^{\cdot\cdot}$ less perfect. These antagonisms were more nearly perfect in the action of ions on the rate of pulsations of medusae, but the anomalous action of $Ca^{\cdot\cdot}$ was again apparent. Perhaps the explanation of this lies in the assumption that the effect of the ions on pulsations is the summation of their effect on various tissue and cell structures, and that the sensitiveness of these structures is different for the same ion, the ions standing in no constant ratio in this regard. This is illustrated by the fact that $Mg^{\cdot\cdot}$ is a strong depressant of muscular contraction whereas Mayer (1915, 1916) has shown it to be without effect on nerve conduction rate.

The All-or-None Law

If the action current (which we may assume is the excitation wave itself) rests on so mechanical a basis as has been set forth it may seem difficult to picture different gradations of excitation. Much evidence is being accumulated by physiologists to show that no gradations of excitation exist so far as the single cell is concerned, but that all excitations are maximal (Bayliss, 1915 a). This is called the all-or-none law. A weak nerve impulse is explained as an impulse in which only a few fibers take part and a maximal impulse one in which all of the fibers take part; and the same principle holds for muscle. Tonic impulses are due to a succession of excitations involving a minute proportion of the fibers at any one time, and producing a fibrillation in the muscle that gives it tone. Such a tonic excitation continues to affect a veratrinized nerve muscle preparation for some time after a single stimulus is applied, thus retarding the relaxation of the muscle. The peculiar series of action currents after veratrin poisoning may be detected with the string galvanometer (Lodholz, 1913). Such a delicate instrument is not necessary in case of the electric organ, however, which shows the same behavior toward veratrin (Winterstein, 1910 iii (2) 185).

The all-or-none law removes a series of objections which might be raised against a purely physicochemical hypothesis of excitation. It is the present purpose merely to call attention to this fact. For a discussion of the evidence in support of the law see Bayliss's Physiology.

Absorption and Secretion

The fact that the intestine may absorb a hypertonic solution introduced into its lumen has for a long time been the cause of astonishment. Hamburger (1908) found this valvelike action to be shown (in a very much smaller degree) by dead intestinal wall and by double membranes made by pasting together two membranes of different material. These results remind us of the theory of Flusin (1908 a) that osmose is controlled by three factors:

1. The attraction of the membrane for the solvent, determining its imbibition.
2. The presence of solutes affecting this imbibition.
3. The mutual attraction between solute and solvent.

This third factor is the principal one according to Traube, who calls it "haftdruck." Assuming 2 and 3 to remain constant, osmose is affected by imbibition of the membrane, and if imbibition is different on the two sides the osmose might be forced in one direction or the other. This seems to be the view of H. N. Morse as a result of his direct determination of osmotic pressure. Osmose is then the resultant of three processes, between the first solution and the membrane, between the two sides of the membrane, and between the membrane and the second solution.

It seems evident that one effect of imbibition is to alter the size of the pores, and Bartell has shown that the enlargement of the pores can change positive into negative osmose. Electro-endosmose seems to be at least one factor in the valvelike action of living membranes. Girard (1910) observed that the valvelike action of frog's skin separating isotonic salt from sugar solution, ceased when the skin current was inhibited by anesthetics.

Assuming that living membranes are easily permeable to H ions, the more acid of two solutions separated by the membrane should be electronegative, and the osmose should be toward it, since electroendosmose is usually toward the negative pole. This view is in harmony with the fact that osmose is from the alkaline blood to the acid stomach and kidney cavities, but from the alkaline intestine to the slightly less alkaline blood. It is probable that protoplasm is less alkaline than blood, hence the turgidity of cells.

Bernstein in his "Elektrobiologie" accords first place to electro-endosmose in explaining osmose through living membranes. The electric polarization of such membranes is reduced or disappears on death—hence, electroendosmose must be reduced or cease also. The cause of this cessation of electroendosmose at death is probably not a sudden abolition of the difference in concentration of ions causing the emf, but an alteration of the membrane. It was shown long ago by Nägeli, and repeatedly confirmed by others, that increase in permeability of the plasma membrane is so intimately associated with death of the cell that the two phenomena are probably the same thing. This seems true notwithstanding the fact that the plasma membrane may not lose *all* of its semipermeable properties on death. Some of the evidence that disappearance of the bioelectric phenomena alter osmose are as follows:

If the turgor of living cells is due to electroendosmose, dead cells should dry in air sooner than live ones. This fact is the teleological principle involved in the instinct of certain wasps who store spiders with the eggs so that the larvae may have food. If the spiders are killed they dry up before the eggs hatch, hence the necessity of keeping them alive. The wasp stings the spider in its central nervous system so that it remains alive in a paralytic condition. Bernstein ("Elektrobiologie") tied living and dead skin over the mouths of two tubes filled with water. The evaporation took place more rapidly through the dead skin. He suspended two similar pieces of the frog's skin from the two arms of a balance. One of the pieces of skin was killed, and it then lost weight faster than the other. A similar experiment with plant leaves gave the same result. The same was true of frog's muscle if the experiment were terminated within a half hour, at the end of which time the live muscle had been killed by drying.

Recent experiments by F. H. Scott on blood volume and by A. D. Hirschfelder on edema confirm the older ideas that the transfer of fluid in the mammalian body is largely accounted for by filtration and the osmotic pressure of colloids. The osmotic pressure of colloids is considered in Chapter VI.

CHAPTER XI

ANESTHESIA AND NARCOSIS

We are familiar with the power of anesthetics in restraining the activity of the central nervous system, especially the reflex and voluntary activities which they affect in lower concentrations than those affecting other organs. Since the higher animals die as soon as the activity of the respiratory center ceases, and under artificial respiration as soon as the heart beat ceases, it is possible in the intact animal to study the effects of anesthetics only on certain nerve synapses, i. e., structures whose threshold of anesthesia is lower than that of the heart mechanism. Anesthetics in higher concentrations suspend the activity of cells and of animals lacking a nervous system. Claude Bernard showed that ether may prevent the movement of the leaves of Mimosa, a sensitive plant.

Overton (1901) observed that vertebrates, insects, and entomostraca (small crustacea) require practically the same concentration of anesthetic (or the same vapor pressure of volatile anesthetic) in order to suspend reflex excitability, whereas certain groups of worms require double this concentration. Protozoa and plants require, in order to suspend their activities, six times the concentration of anesthetic required to anesthetize vertebrates. From Warburg's data, the concentration of anesthetic necessary to reduce the oxidation of goose erythrocytes 30-70 per cent is about six times the concentration necessary to anesthetize vertebrates, although some variation in regard to individual anesthetics is apparent. Slight discrepancies might be due to the fact that the anesthesia data were taken by Overton on samples of anesthetic of a lower degree of purity than those used by Warburg to reduce oxidation. It follows from this that protozoan and plant cells react to anesthetics, not like nerve synapses but like mammalian cells. According to Winterstein (1914), however, narcosis of frog's spinal cord may either decrease or increase oxidation. O_2 is necessary for complete recovery.

It appears, therefore, that cells in general require about the same concentration of anesthetic to suspend their activities. Since this process seems to be the reverse of stimulation and stimulation is associated with increase in permeability, we might expect anesthetics to prevent increase in permeability. Traube (1908 a) observed that isoamyl alcohol, which belongs to a class of substances which in high concentration cause hemolysis, at a certain low concentration tends to prevent hemolysis of the erythrocytes by other agents.

Lillie (1909 b) observed that pure solutions of certain salts cause an outward diffusion of the pigment from Arbacia eggs, and interprets this as increase in permeability. He also found that anesthetics inhibit this action of the salt (1914, b), so that the salt solution + anesthetic may not cause loss of pigment. This is in harmony with the recent observation of S. Loewe (1913) that anesthetics decrease the electric conductivity of lipoid membranes soaked in salt solution. These results are probably due to increase in size of colloidal lipoid particles. Clowes (1916) supposes the plasma membrane to be an emulsion of lipoid in water that is changed by anesthetics to an emulsion of water in lipoid.

Osterhout (1913 a) observed that small quantities of anesthetics added to the sea water decrease the electric conductivity of seaweed. He had already observed that Na increases the conductivity and Ca decreases it. Since Ca and anesthetic both inhibit the conductivity increasing action of Na, it seems probable that the anesthetic in the sea water inhibits the action of the Na already in the sea water. It follows from this that anesthetics inhibit the increase in permeability to ions.

Hemolysis consists in the loss of the hemoglobin from the red blood corpuscles. Not only does the permeability of hemoglobin increase but Stewart (1899) has shown that the permeability to ions increases. Arrhenius and Bubonovic observed that anesthetics inhibit hemolysis by hypotonic solutions. Joel (1915) showed that anesthetics inhibit the permeability increasing action of pure cane sugar solution on erythrocytes.

The eggs of certain fish are admirable for permeability studies, since they develop in distilled water, in which they neither swell nor lose salts. It had been observed that the permeability of

Fundulus eggs may be increased by certain solutions of salts, alkaloids or other substances in concentrations that do not kill them (McClendon, 1912 a, e, 1913 a). The eggs of the pike behave similarly. These eggs are impermeable to water or to salts, but when placed in 0.1 mol $NaNO_3$ they lose chlorides, which pass out into the nitrate solution and may be accurately estimated with Richards's nephelometer. Three per cent ethyl alcohol causes retardation in the development of these eggs and is therefore considered an anesthetic. If the eggs are placed in 0.1 mol $NaNO_3$ containing 3 per cent alcohol only half as much chlorides diffuse out of them as when placed in 0.1 mol pure $NaNO_3$. The anesthetic inhibits the permeability increasing action of the pure salt solution (McClendon, 1914 b, 1915 b).

The skin current of the frog disappears when the skin is anesthetized, indicating that the hypothetical increase in permeability of the outer surfaces of the gland cells during secretion may be inhibited by anesthetics. In this case we do not know whether nervous stimulants are exclusively the cause of the increase in permeability.

It seems clear, therefore, that anesthetics inhibit the increase in permeability of the plasma membranes of the irritable elements. In order for them to do this it is necessary for them to penetrate only the surface film of the cell. But anesthetics belong to the group of substances which penetrate cells easily, and the question arises as to their effect on the interior. Some experiments on catalysis are suggestive. If blood charcoal is mixed with a solution of oxalic acid containing atmospheric oxygen the acid is burned to CO_2 and H_2O. Blood charcoal contains mineral matter, including iron, which is not entirely removed by extraction with HCl and we cannot say that it is the carbon that acts as the catalyzer. Warburg found that the catalyzing power of charcoal varies with its power of adsorbing oxalic acid, hence the combustion must take place near the surface of the charcoal. He observed further that anesthetics (of the urethane series) inhibit the oxidation, and explained this as due to the displacement of the oxalic acid from the surface of the charcoal by the anesthetic (which is adsorbed to a greater extent than the acid). The author confirmed these results, using charcoal extracted with concentrated HCl followed by H_2O for many hours. This charcoal was heated to redness and cooled in a CO_2-free atmosphere. The charcoal adsorbed all of the CO_2 produced for a time, but after it had become saturated with this gas, the volume of O_2 used up was half the volume of the CO_2 given out. No food substance was found that could be burned in this way. Rona and Tosh (1914) found, however, that urethane displaces adsorbed dextrose from animal charcoal.

Meyerhof (1914 b) observed that the catalysis of hydrogen peroxide by colloidal platinum may be inhibited by anesthetics. Since the anesthetic did not reduce the number of particles of platinum seen in the ultramicroscope he concluded that the anesthetic displaced the H_2O_2 from the surface of the platinum.

Meyerhof (1915) showed that solution of invertase from yeast may be inhibited by anesthetics. The following table shows the concentration of anesthetic that reduced the inversion of 3 per cent cane sugar solution 30 per cent:

Methyl alcohol	3 mol
Ethyl "	1.3 "
Propyl "	.5 "
Isobutyl "	.27 "
Isoamyl (fermentation)	.21 "
Methyl urethane	.95 "
Ethyl "	.97 "
Propyl "	.48 "

In this case the Tyndall phenomenon was increased, but this may have been due to an increase in the dispersion of light by particles, due to their inclosure in films of anesthetic, rather than an increase in the size of the particles. In harmony with the view that the reduction in activity of the enzyme is due to the displacement, by the anesthetic, of the sugar from its surface, is the fact that if the concentration of the anesthetic is varied its effectiveness follows the typical adsorption curve (Fig. 24). The concentration of anesthetic necessary to anesthetize tadpoles reduced the inversion about 20 per cent, but this reduction was increased by dilution of the sugar solution.

If the anesthetics are added in greater concentrations to enzyme-containing extracts of cells, precipitation may occur. The enzymes are carried down in the precipitates, but it is not clear whether the enzyme is precipitated directly by the anesthetic, or protein impurities precipitated and the enzyme adsorbed by the protein. Batelli and Stern (1913) showed that anesthetics precipitate nucleoprotein.

Warburg and Wiesel (1912) observed that anesthetics inhibit the fermentation by yeast juice, attributing this inhibition to the precipitation of the zymase, since the inhibiting and precipitating powers of anesthetics are parallel. However, live yeast requires for inhibition less concentration of anesthetic than is required by yeast juice (or for precipitation of protein).

Vernon (1912 a) observed that anesthetics are harmless to the indophenol oxidase of chopped kidney until a certain concentration is reached, above which they inhibit its action. The data of Batelli and Stern are given in the following table, as compared with the anesthetization of tadpoles by Overton and the reduction of the respiration of sea urchin eggs, fish sperm, goose erythrocytes, lymphocytes, liver cells, spinal cord, yeast and bacteria 30-70 per cent, by Warburg. The figures are the gram molecular concentrations:

	Inhibition of oxidation at 15°.	Precipitation of nucleoproteid.	Anesthesia of tadpoles, Overton.	Inhibition of respiration of cells, Warburg.
Methyl alcohol	11.44	12.48	0.62	5
Ethyl alcohol	6.12	6.97	0.31	1.6
Propyl alcohol	2.04	2.72	0.11	0.8
Isobutyl alcohol	1.06	no ppt.	0.045	0.15
Isoamyl alcohol	0.41	no ppt.	0.023	0.045
Acetone	5.70	4.34	0.26	
Methylethylketone	2.22	2.41	0.09	
Methylpropylketone	0.77	no ppt.	0.019	
Allyl alcohol	2.70	4.05		
Ethyl urethane	1.90	2.58	0.037	0.33

This table shows that about six times the concentration of anesthetic effective on tadpoles is required to inhibit the respiration of goose erythrocytes. Gros (1910) found the same thing true for the anesthesia of peripheral nerves. It seems, therefore, that the nerve cells of the tadpoles are not anesthetized, but the synapses are in the same condition as during sleep. For anesthetization of nerve cells and reduction of the respiration of goose erythrocytes the same concentration is required, but for inhibition of the oxidases and precipitation of protein in Batelli and Stern's experiments more than double this concentration is needed. In fact, the concentration used by Batelli and Stern is much above the lethal dose for cells. Warburg (1911 a) explains the fact that anesthetics inhibit enzymes, when contained in living cells, in lower concentrations than the same anesthetics inhibit the same enzymes in cell extracts, by the assumption that the enzymes within the cells are adsorbed to phase boundaries, the anesthetics becoming more concentrated at the phase boundaries. If this explanation is correct we must make the further assumption that the enzymes in the cell extracts referred to are in true solution, whereas the invertase used by Meyerhof formed a colloidal solution because the invertase was inhibited by as low a concentration of anesthetic as is required to inhibit the respiration of the erythrocytes.

Although Batelli and Stern claim that anesthetics precipitate proteins Traube and Köhler (1915) and Traube (1915) assert that anesthetics help in the solution of proteins. In the following table they have arranged anesthetics according to their power of hastening the swelling and solation of gelatine jelly (and retarding gelation of sols). It is found that they are with few exceptions in the order of anesthetic power, as shown by the figures in the last column, which represent the molecular concentration for anesthesia.

Anesthetic	Anesthetic concentration (Overton)
Phenanthren	.0000037
Thymol	.000055
Napthaline	.000065
Chloroform	.0014
Chloral hydrate (acid)	.006
Ethyl ether	.001
Ethyl chloride	.0045
Sulfonal	.0088
Trional	.0064
Isoamyl alcohol	.023
Ethyl urethane	.041
Isobutyl alcohol	.045
Methylethyl ketone	.09
Ethyl acetate (acid)	.03
Proprionitrile	——
Tert. amyl alcohol	.057
Propyl alcohol	.11
Acetone	.26
Ethyl alcohol	.39
Methyl alcohol	.57

Perhaps the anesthetics protect the gelatine from the precipitating action of accidental impurities. On the contrary, if impurities hold a protein in solution the anesthetic might then help precipitate the protein. This may explain the opposite effect of anesthetics on nucleoprotein solutions.

This action of anesthetics in displacing other substances from the surface film probably explains the observation of Abl (1907) that they reduce the electrolytic solution tension of cadmium, and the observation of Grumbach (1911) that they reduce the contact potential between dielectric and electrolyte.

The action of anesthetics on living cells may be explained by the same assumption, i. e., that they cover the plasma membrane with a film which protects it from change, entering the cell and displacing enzymes or reacting substances from the phase boundaries where these reactions occur more rapidly than elsewhere. The synapses that have to do with consciousness are more sensitive to anesthetics than are either plasma membranes or these enzyme reactions. Hence, it requires only one-sixth the concentration to anesthetize that it requires to inhibit other processes.

This hypothesis also explains the relation between anesthetic power and surface tension as emphasized by Traube, who states that the more an anesthetic lowers the surface tension of water the greater is its anesthetic power. This is nearly correct, due to the fact that anesthetics that lower the water-air surface tension are adsorbed to many water-solid or water-liquid surfaces.

According to Overton (1901) and H. Meyer (1901), anesthetic power varies with the partition coefficient of the anesthetic between lipoid and water. This partition coefficient has never been determined, but these authors have been content with the partition coefficient between olive oil and water or between some solution of lipoid and water. In many cases even this was not determined, but the ratio between the solubility in lipoid solution and in water substituted. Owing to the concentration at the phase boundary, these two magnitudes are probably never the same. But it seems generally true that substances which very greatly lower the surface tension between water and air, have a high partition coefficient between oil and water and are therefore soluble in oil—they are also soluble in many other organic solvents. The "solubility" of an anesthetic in lipoid "solutions" seems to be due to the fact that it is adsorbed by the colloidal particles of lipoid so that the solution can hold more anesthetic than the solvent (benzol, etc.) can. Thus we see that adsorption is at the basis of the whole matter.

In some cases the partition coefficient of the anesthetic between cells or tissues and water has been determined. Warburg, by this means, concluded that the erythrocytes consist of a larger volume of watery phase than of lipoid phase.

It has been shown by a number of investigators that alcohol, ether, chloroform, chloral hydrate and acetone become more concentrated in nerve than in other tissue, this being used to explain the ease with which the central nervous system may be anesthetized. The nervous system, however, stores the anesthetic partly in lipoids which are more abundant in the white matter, forming the myelin sheaths of the nerves, and the nerves are no more easily anesthetized than non-nervous cells (Gros). Nerve impulses are still carried in the anesthetized body, only certain nervous processes being stopped, as in sleep. This stoppage may be explained by the assumption that certain synapses are affected by the anesthetic.

It is characteristic of pure anesthesia that it is a reversible process, brought about only by a certain concentration of anesthetic. Many anesthetics, even in the optimum concentration, have not only an anesthetic but also a toxic action, so that prolonged anesthesia is harmful. This is characteristic of all anesthetics with acid or basic properties, the alkaloids, for instance, whose chemical affinity may explain their selective action on certain cell structures. Other anesthetics are decomposed in the body into harmful substances. Esters are saponified with the liberation of acids.

But even the purest types of anesthetics are toxic if the anesthetizing dose is exceeded (provided they are sufficiently soluble to exceed this dose). This toxic action is manifested by increase in permeability, which is reversible only if very slight and momentary. The action of anesthetics in these high concentrations will be considered in the chapter on antiseptics and in other chapters.

When ether is given to an animal, a stage of excitement sometimes precedes that of narcosis. This fact is open to various interpretations in an animal with a cerebrum, but Hamburger (1912) observed that ameboid motion is increased by minute doses of anesthetics. Johannsen (1906) observed that lilacs sprout earlier after treatment with ether vapor. Further examples of the stimulating action of anesthetics are given by Carlson (1906), Vernon (1912), Tashiro and Adams (1914), Lee (1903), and Lee and Salant (1902). The possibility is not excluded, therefore, of three concentrations of anesthetics producing three kinds of responses: first, stimulation; second, narcosis, and third, violent stimulation causing death if continued.

The following table (modified from Lillie, 1916 c) summarizes the effect of anesthetics (alcohols) on various processes, giving the effective mol concentration.

Effect	Methyl	Ethyl	Propyl	Butyl	i-Amyl
Narcosis of tadpoles (Overton)......	0.57	0.29	0.11	0.038	0.023
Phototropism of Daphnia (Loeb).....	0.6	0.2	0.075	0.04	—
Inhibition of sea urchin eggs (Fühner)	0.72	0.41	0.136	0.045	—
Retardation of fish eggs (McClendon)	—	0.52	—	—	0.015
Half rate of turtle ventricle (Vernon)	1.1	0.53	0.23	0.05	0.02
Narcosis of Daphnia (Loeb)..........	1.2	0.6	0.2	0.12	0.04
Inhibition of sea urchin eggs (Lillie)..	—	0.87	0.27	0.086	0.037
Narcosis of worm larvae (Lillie).....	2.2	1.1	0.34	0.09	0.03
Depression of invertase (Meyerhof)...	3.0	1.3	0.5	0.27	0.21
Half oxidation in erythrocytes (Warburg)	5.0	1.6	0.8	0.15	0.045
Ppt. nucleoproteid (Batelli and Stern)	5.7	2.38	1.12	0.45	0.21
Inhibition of ether-yeast (Warburg)..	5.0	3.5	1.3	0.54	0.23
Kills muscle oxidon (Batelli and Stern)	7.54	3.57	1.16	0.44	0.19
Kills indophenol oxidase (Vernon)....	12.0	6.4	3.1	0.61	—
Reduce surface tension to 0.5.........	14.0	5.0	1.6	0.46	0.14

CHAPTER XII

CYTOLYSIS AND DISINFECTION

The first action of many toxic substances on cells is the destruction of the plasma membrane. The cell is then no longer influenced by the osmotic pressure of the medium but may swell or shrink as any other colloid mass, depending on certain conditions. Animal cells after destruction of the plasma membrane usually swell and disintegrate, a process known as cytolysis, meaning the dissolution of the cell. It seems probable that death of all cells is accompanied by cytolysis, but that other processes sometimes mask the cytolytic changes. Thus, in the histological fixation of cells the protoplasm is transformed into a stable mass immediately after the destruction of the plasma membrane, so that no swelling or shrinking follows the increase in permeability. Since cytolysis means death of the cell, all cytolytics may be used as antiseptics, destroying at least the active forms of microorganisms.

The indifferent anesthetics, when their concentration exceeds that necessary for the inhibition of cell activities, increase the permeability of the plasma membrane. This increase, if slight and momentary, may be reversible, at least in some cases. If the semipermeability of the cell is entirely removed, cytolysis and death occur. For this reason anesthetics have an antiseptic action. Some active cells are more resistant to anesthetics than others, the spores of bacteria usually not being killed, but merely prevented from germinating so long as the anesthetic is present.

The fact that anesthetics increase the permeability of cells is illustrated by the fact that they accelerate autolysis. If tissue is cut off from the blood supply, either by ligature of the vessels or by removal from the body, and kept free from germs, it digests itself, a process known as autolysis. Certain substances accelerate autolysis. Chiari (1909) observed that ether accelerates autolysis. Hans Meyer (1909) explained its action as an increase in permeability of the tissue, allowing the contact of protease with protein and a general diffusion and mixing of the substances in the tissue.

The cytolytic and antiseptic power of anesthetics varies with their power of penetrating into the plasma membrane substance, and hence varies approximately with their power of lowering the surface tension of water. Solutions of the same surface tension are called isocapillary solutions. It was found that isocapillary solutions of ether, alcohols, and acetone just caused death of Fundulus eggs, with increase in permeability (McClendon, 1912 a). Czapek (1910) observed that indifferent anesthetics just caused death and increased permeability of plant cells when they lowered the surface tension of water to .68. Scheurlen (1896) observed that NaCl increases the toxicity of phenol. This may not be an additive effect, as NaCl may reduce the surface tension of phenol solutions in the same way that it reduces the surface tension of bile (Allen, G. D., 1915 a).

It seems, therefore, that the toxicity of all indifferent anesthetics depends on the concentration and that the concentration of the anesthetic in the plasma membrane is the only variable. With other substances, however, this is not the case. If the anesthetic is of such nature as to be chemically active in the ordinary sense (not through residual valences) it is more toxic than should be from the surface tension of its solution. Substances with basic, acid, reducing or oxidizing properties are especially toxic, ammonia, phenol, formaldehyde and H_2O_2 being examples. But the acidity of phenol is not high enough to account for its toxicity, and formaldehyde, besides having a reducing action, forms additive compounds with protoplasm in the same way that it combines with ammonia to form hexamethylentetramine.

The toxicity of cations varies inversely with the electrolytic solution tension, therefore the following series arranged in decreasing order of solution tensions is for the most part in increasing order of toxicity: Cs, Li, Rb, K, Na, Ba, Sr, Ca, Mg, Al, Mn, Zn, Cd, Sn, Fe, Tl, Co, Ni, Pb, (H), As, Cu, Bi, Sb, Hg, Ag, Pl, Pt, Au.

Mathews (1904 a) found the order of toxicity of salts of these ions on Fundulus eggs to be Mn, Fe, Co, Ni, Zn, Pb, Cd, Cu, Hg, Ag, Au. It may be noticed that Zn and Cd are out of the place they occupy in the series of electrolytic solution tensions. The $ZnCl_2$ used was more toxic than it should be, owing probably to hydrolysis, by which the solution became acid. The great toxicity of the $CdCl_2$ is probably not due entirely to the cadmium ion but partly to the unusually large proportion of undissociated molecules which are easily adsorbed.

In estimating the toxicity of ions, the degree of ionization must be calculated. The addition of large quantities of some electrolytes to solutions of some heavy metals inhibits the ionization of the latter by the formation of double salts. If NaCl is added to $HgCl_2$ solution, Na_2HgCl_4 is formed, which is very poorly dissociated by steps, forming: $Na^{\cdot}$ and $NaHgCl_4{}'$, $Na^{\cdot}$ and $HgCl_4{}''$, $Hg^{\cdot\cdot}$ and $4Cl'$. The number of Hg ions formed is very small and the addition of quantities of NaCl reduces it to a vanishing magnitude.

The addition of NaCl increases the solubility of $HgCl_2$ but not the concentration of $Hg^{\cdot\cdot}$. If solid $HgCl_2$ is shaken with water, the salt dissolves until there is an equilibrium between the solid phase and the undissociated molecules in solution. Some of these molecules dissociate, thus disturbing the equilibrium, and more of the solid dissolves to restore it, until a second equilibrium is established between the ions and the molecules. This equilibrium is expressed by the equation $\frac{[Hg^{\cdot\cdot}]\times[Cl']}{[HgCl_2]} =$ a constant, but since the concentration of the undissociated molecules is constant so long as there is any undissolved salt, the equation becomes $[Hg^{\cdot\cdot}]\times[Cl'] =$ a constant. If we add Hg $(NO_3)_2$, thus increasing the concentration of Hg ions, this solubility product will be exceeded and some $Hg^{\cdot\cdot}$ and Cl' will combine, the additional $HgCl_2$ thus formed crystallizing out. If, on the contrary, we add NaCl, the Cl' will in the same way lower the solubility of Hg ions, but the $Na^{\cdot}$ combines with the Hg and Cl ions forming Na_2HgCl_4, thus removing them, more $HgCl_2$ dissociates, and more bichloride dissolves. This process is continued until the solution is saturated with Na_2HgCl_4. The concentration of $HgCl_2$ at equilibrium is the same as before, but the concentration of Hg ions decreases when the concentration of Cl ions increases, since their product is constant. The solution loses most of the Hg ions if the concentration of the NaCl is high, but gains the Na_2HgCl_4, which more than compensates this loss, so that the total Hg in solution is greater than before.

Although the solubility of $HgCl_2$ is increased by the addition of NaCl, and the mercury may be used in greater concentration, this does not mean that the toxicity is increased. The question as to whether the ions or molecules are the more toxic must first be answered. This seems to vary with the duration of the exposure to the poison, as shown by the following experiments:

Paul and Krönig (1896) determined the disinfecting power of differently dissociated salts of mercury on Anthrax spores. Those salts which are poorly dissociated are less toxic than those which are more completely dissociated. In other words, the toxicity depends chiefly on the mercuric ions. The following table shows the number of colonies that developed after exposure to the toxic solutions:

Salt concentration	After 20 min. exposure	After 85 min. exposure	After 90 min. exposure
$HgCl_2$ 1/64 mol	7 colonies	0 colonies	
$HgBr_2$ 1/64	34	0	
$HgCy_2$ 1/16	00	33	
K_2HgCl_4 1/16			0 colonies
K_2HgBr_4 1/16			5
K_2HgI_4 1/16			389
K_2HgCy_4 1/16			1035

The same rule had previously been found by Kahlenberg and True (1891) to apply to the toxicity of copper salts, and seems to be approximately true of all heavy metals. It is only approximately true because the molecules are toxic, although not so toxic as the ions, during brief periods of exposure, and the rule does not hold for prolonged exposure.

If salts of heavy metals are allowed to act on organisms for a long time, their toxicity is approximately proportional to the total quantity of the heavy metal they contain. Stevens (1898) and Clark (1899) grew Penicillium and Aspergillus in such solutions, finding that the inhibition of growth is approximately proportional to the quantity of the heavy metal, without regard to the combination in which it exists. It is probable that the heavy metal ions combine with the protoplasm, thus leading to further dissociation of the salt until all is dissociated and combined.

This seems to be the action of organic preparations of heavy metals such as "argyrol."

The toxicity of each cation may be decreased more or less by certain other cations. For this reason, the toxicity of a salt is reduced when it is added to solutions containing other salts (as is always the case where it is desired to disinfect). Only the most toxic salts, therefore, are reliable for disinfection. Copper salts may be used in some cases but salts of mercury are better. The salts of the precious metals are the most reliable.

The salts of the lighter metals may be toxic, especially if their solutions are pure and kept pure by replacing the contaminated solution with fresh ones. Some discrepancies seem to exist between the solution-tension series and the toxicity series, but these would probably be lessened if all of the salts were absolutely pure and if some indifferent substance could be found to equalize the osmotic pressure. Höber (1908 a) studied the relative efficiency of slightly hypotonic solutions in causing cytolysis of erythrocytes (hemolysis). The ionic series with increasing hemolytic power is as follows:

$$SO_4 < Cl < Br < NO_3 < CNS < I \text{ and}$$
$$Li, Na < Cs < Rb < K$$

Lillie (1909 a) observed the same anionic series for the cytolysis of sea urchin eggs, but $Na^{\cdot}$ was more toxic than $K^{\cdot}$. The same anionic series was found to hold for the cytolysis (dissolution) of cilia (Lillie, 1909 c; Höber, 1909 b), but the cationic series is different for different species of animals. In general, it may be said that the iodides of the alkali metals, when pure, exert a cytolytic action.

It has been shown by Kahlenberg and True (1896) and others that the toxicity of strong acids depends on the concentration of the hydrogen ions. Mineral acids cause death of seedlings and cytolysis of many plant cells when the concentration exceeds 1/6400 normal. For disinfection .1n or stronger solutions may be used.

The toxicity of the weak acids depends on several factors, so that no general rule may be made in regard to them. Their power of reducing the surface tension of water is the index of one factor. Acetic acid and sulphurous acid are used in disinfection.

The toxicity of the strong bases depends on the hydroxyl ion concentration. These are seldom used for disinfection because they are neutralized by the CO_2 of the air. Quick lime is used more for its drying power and the toxicity of the Ca ions, than for the toxicity of the OH ions.

The weak bases are similar to the weak acids in that their power of reducing the surface tension of water must be considered. Some of them, such as quinine and strychnine, are especially destructive to certain protozoan parasites.

Solutions of soap are alkaline, due to hydrolysis. Soap lowers the surface tension of water enormously and has a marked cytolytic action. It destroys the free forms of many bacteria.

We may summarize some of the disinfectants as follows:

Anesthetics.	Alcohol, 50-70%. Phenol 5%, 24 hrs.; Cresol 5%, 2 hrs.
Oxidizers.	H_2O_2 1%, 30 min. Cl_2; Br_2; I_2.
Reducers.	Formaldehyde, SO_2.
Strong acids.	0.1 normal 15 minutes.
Weak acids.	Acetic, concentrated.
Strong bases.	$Ca(OH)_2$ (dehydrating).
Weak bases.	Quinine.
Soaps.	
Heavy metals.	$HgCl_2$; Cacodylic acid $AgNO_3$ Argyrol; $CuSO_4$; Salvarsan.

CHAPTER XIII

AMEBOID MOTION AND TROPISMS, CELL DIVISION, FERTILIZATION AND PARTHENOGENESIS

It was shown by Quincke that movements of drops of fluid, resembling ameboid motion, could be produced by localized changes in surface tension. A local reduction in surface tension produces a protrusion of the affected surface—a localized increase produces a flattening. By a combination of such changes in surface tension, combined with adhesions to the substratum and to food particles, Rhumbler (1905, 1910) has imitated all forms of ameboid motion.

In imitating food taking, Rhumbler found that his artificial amebae would take only such food as adhered to them. A chloroform drop would engulf a piece of shellac but not take a piece of glass. If a piece of glass is coated with shellac, the chloroform drop will engulf it, but after the shellac is dissolved off it will reject the glass. In this way the process of defecation by the ameba is imitated. Such a process also imitates the shell building of some protozoa. These shells are formed of defecated particles which cover the surface of the protozoon. If chloroform is shaken with powdered glass and dropped into water, the drops are found to be surrounded by shells composed of particles of glass.

The ameba apparently does not adhere to all of the food that it takes, shown by the fact that it often takes in a quantity of water with the food particle. The food is separated from the protoplasm on all sides by water, and thus, after ingestion, comes to lie in a food vacuole. Bernstein (1900) was able to make an artificial ameba take food to which it did not adhere. A drop of mercury was immersed in dilute nitric acid and a crystal of potassium bichromate held near it. As soon as some dissolved bichromate reached the mercury the surface tension was reduced and the mercury moved toward the crystal. The momentum of the mercury was often so great that it engulfed the crystal. This experiment is crude, the movement being very rapid and the crystal immediately thrown out after being ingested.

In order to explain the food taking of the ameba, a still further localization of surface tension changes must be assumed. If we assume that a substance diffuses from the food, which in very low concentration causes a reduction in surface tension of the ameba, but in higher concentration causes an increase in the surface tension, we can explain this form of food taking. When the ameba comes within the sphere of influence of the food its surface tension is reduced on that side, and it advances or sends a broad pseudopod toward the food. The center of the advancing edge of the ameba comes so close to the food that its surface tension is increased and its forward motion is stopped. On each side of this central area, the protoplasm continues to advance and finally forms a ring around the food. The upper and lower edges of this ring constrict and thus enclose the food together with some water in a hollow sphere of protoplasm.

Quincke (1888 a) supposed the surface of protoplasm to be of an oily nature, and hence soap should lower the surface tension. It was found, however, that soap, no matter what the concentration, always increased the surface tension of the ameba, when it had any effect at all (McClendon, 1911 c). Since no phase boundary is known whose tension is increased by the presence of soap in either phase, some indirect action of the soap must be looked for.

Lippman showed that electrical polarization of a phase boundary reduces the surface tension, and that reduction of this polarization increases the surface tension. We have seen that there is abundant evidence to show that the plasma membrane of living cells in the resting condition is electrically polarized, and that increase in permeability reduces this polarization. Increase in permeability, therefore, increases the surface tension of cells. This is demonstrated by the rounding up of an ameba when it is strongly stimulated. If this stimulation is accompanied by increase in permeability, as in the case of certain plant cells, and apparently in the case of the muscle cell, we should expect a reduction of the electrical polarization, increase in surface tension, and an approach toward the spherical form.

That the surface of the ameba is electrically polarized is shown by the fact that the ameba moves toward the anode in an electric field, but this fact does not prove whether the electrical polarization is due to semipermeability or to the electrical polarization of the colloidal particles of which the surface of the ameba is composed. The movement in the electric field (electric convection) may be abolished, or reversed, by adding H ions or certain heavy metal ions to the medium. These ions influence the permeability as well as the electric polarization of colloidal particles. The former is certain, and it is simplest to assume that the change in polarization is the result of the change in permeability alone, and not the combined effect of change in permeability and adsorption. The permeability hypothesis explains a number of physiological processes and is in harmony with a larger number of facts than any other.

All of those agents which stimulate muscle or nerve cause a rounding up of the ameba. We may interpret this approach to the spherical form as due to increase in the tension of the plasma membrane, following decrease in polarization, which is the result of increase in permeability or the disappearance of the semipermeability to certain ions or classes of ions. We should, therefore, expect most of the tropisms of the ameba to be negative, as is found to be the case. The ameba avoids the region from which a strong stimulus comes because the permeability and surface tension of the plasma membrane on that side is increased and the protoplasm flows in the opposite direction.

It is more difficult to explain positive tropisms, since decrease in permeability has been less often measured. But permeability being a relative term, decrease in permeability in one region has the same effect as increase in permeability in all other regions. Osterhout found that the permeability of kelp in calcium salt solutions is less than in sea water. This may be due to the fact that the sodium of the sea water increases the permeability and that the excess of Ca antagonizes the action of the sodium in the sea water, preventing this increase and making it appear that the calcium decreases it. We may assume that salts or other substances in the water tend to increase the permeability and that substances which antagonize their action cause positive tropisms.

The action of an electric field on an ameba on a substratum is in harmony with this view. The anions within the ameba are massed on the inside of the plasma membrane toward the anode because they cannot pass through it. When they have accumulated to a sufficient concentration they act as a stimulus, increasing its permeability, and the ameba moves toward the cathode. This is not due to electric convection, since a suspended ameba is carried toward the anode by this means, electric convection being anodic whereas galvanotropism is cathodic.

We may explain the irregular movements of the ameba when not under a single directive stimulus as due to varying and localized influences of the environment on its permeability, or to localized variations in its metabolism, resulting in variations in the production and concentration of the electrolyte responsible for the electrical polarization.

Certain substances dissolved in the medium increase and others decrease ameboid movements although the mechanism of their action is not known. Hamburger (1912) observed that anesthetics in certain low concentrations increase the ameboid motion of leucocytes. He found that a saturated solution of iodoform had the right concentration, and hence its efficacy in salves in increasing phagocytosis. This explanation rests on the assumption that the phagocytic action of the leucocytes varies with their ameboid activity. Substances which increase their phagocytic action were called opsonins by Wright, but whether such substances have their primary effect on the leucocytes or the bacteria seems undecided.

Ross (1911) claims that alkaloids increase ameboid movements.

The idea that gelation and solation, and swelling and shrinking of gels are factors in ameboid movements was first expressed by Montgomery (1878, 1881), being more recently advanced by Rhumbler and others.

The contractile vacuole seems to be an organ of defense of the ameba against the swelling action of the hypotonic medium. Hartog has shown that it is universal among fresh water amebas but may be absent in parasitic and marine forms. It may be remarked, however, that even some phagocytes in exudates have contractile vacuoles.

Rhumbler (1898) made a model of a contractile vacuole in the following manner. A drop of chloroform (not freed from alcohol, and that had become acid) was immersed under water. A cloud of water droplets appeared within the chloroform and fused to one drop which burst.

The word tropism if used in strict accordance with its derivation would be confined to the multicellular and syncytial plants, but it has been extended to unicellular plants and to animals as well. For instance, the roots of plants are influenced by gravity. If moved out of the normal relation to gravity the tip bends back until the normal relation is regained. Starch grains in certain cells near the root tip are acted on by gravity and seem to act as otoliths stimulating the cells when the root is placed in an abnormal position.

Engelmann (1882) showed that the swarm spores of many green plants are influenced in their movements by external agents. He called these reactions to the environment tropisms, although the reaction is not a growing into a new position, but a turning movement or change in the direction of swimming.

Loeb (1889) has attempted to show that the tropisms of animals are the same as those of plants. This is largely a philosophical question, since the tropisms of plants are very imperfectly understood, and there is no consensus of opinion as to the dividing line between plants and animals.

Loeb distinguishes tropisms and sensibility to change, defining tropisms as the direct action of the environment on the motor organs, producing a direct and correct response. It should be added, however, that he also considers the reactions of certain insects to light as tropisms but cannot assume that the light acts directly on the muscles, the distinguishing characteristic being that the movements are forced. Even the behavior of vertebrates is sometimes described as tropism. Selachians do not seem to possess much intelligence since they butt their heads to pieces on the glass of aquaria until they die. All of their reactions are not simple, however. The author has often observed the rays of Mission Bay, San Diego, Cal., escape after being discovered. The ray moves along the bottom leaving a trail of muddy water, then doubles under this curtain and finally darts out at a new place.

Jennings (1906) has shown that the movements of certain protozoa, that had been considered tropisms, are complex activities in which sensibility to change takes a leading part. Paramecium, when stimulated sufficiently by a change in the environment, reverses some of its cilia, so that it moves a short distance backward and the spiral course of its movement is widened. Being removed from the acute stimulation, it starts forward again from some segment of the spiral. The fact that the spiral is widened during the backward movement causes the course to be altered when the forward course is resumed. If this new course leads to a second stimulation the whole process is repeated. This repetition is continued until the animal ceases to be stimulated. Jennings calls this a case of "trial and error," thus showing a relation to the reactions of man. The distinction between tropism and trial and error is a philosophical one. There is no trial in the teleological sense and the cause of error will not be known until the mechanism of the reaction is understood. Trial and error has the same significance as chance in the laws of chance. Chance is merely a name to express ignorance of unknown factors. Only where these factors have some constant numerical relation to one another, is there a law of chance.

The question arises whether in Paramecium the stimulus acts directly on the motor organs, cilia. Since any strong stimulus, no matter from what direction, causes the same motor response, it would seem that there is some coordinating mechanism for the ciliary movement, or at least that the stimulus is propagated over the body of the Paramecium. Since the stimulus is propagated over the cells of sensitive plants without a special conducting mechanism, there is no reason to suppose that the same thing might not be true of animals. Nemec describes a conducting mechanism in plants, but this has been shown to be an artefact (McClendon, 1910 b). Neresheimer describes a conducting mechanism in infusoria, but this also is doubted.

The ameboid movements just described might properly be classed as tropisms, but even in the ameba there seems to be some evidence of the transmission of stimuli over the plasma membrane (McClendon, 1909 a).

The reactions of protozoa to constant electric currents seem to harmonize with the idea that tropisms are forced movements.

Galvanotropism cannot be explained away as electric convection since the galvanotropism of the ameba is cathodic, whereas the electric convection of the ameba that is unable to reach and catch hold of the substratum is anodic. Ludloff (1895) showed that the galvanotropism of Paramecium is a forced movement, the cilia toward the cathode being reversed, and causing a turning toward the cathode. Since any strong stimulus causes reversal of cilia, this observation shows that the paramecium is stimulated at the cathode, even by a constant current. Dale (1911) has studied galvanotropism in a number of species of infusoria.

The direction of tropisms has been reversed by change in viscosity, reaction, or temperature of the medium, and by the addition of neutral salts, acids and anesthetics. Some reagents employed, such as CO_2 and alkaloids, change the reaction but their action may not be due to H ions. Wo. Ostwald supposes that a change in viscosity that reduces the efficiency of the motor organs changes negative geotropism into positive geotropism. If this is true, the head of the animal must already be pointed away from the earth, in order that positive geotropism take place. Furthermore, negative geotropism would be analogous to the effect of a strong constant electric current which carries the paramecium to the anode while its head is toward the cathode. Such a change from positive to negative geotropism is not what is usually meant by a reversal of tropism, which is made to imply a reversal of orientation. Some writers use the word taxis since it means orientation, but tropism, meaning a growing and borrowed from plant physiology, has come into general use.

Bancroft (1906) attempted to identify reversal of tropisms of Paramecium with the antagonistic actions of Ca to Na, claiming that the direction of tropism is determined by the ratio of Ca to Na.

We have attempted to explain ameboid motion, and consequently tropisms of the ameba as due to local increase in permeability. The ameba recedes from the side on which the permeability is increased. The agent causing positive tropism must therefore shield the ameba from stimuli. The movements of plants are caused by increase of permeability, and Tröndle (1910) and Lepeschkin (1908) have shown that light increases the permeability of plants to salts and dextrose.

The geotropism of roots has been supposed to be due to the action of starch grains in the cells as statoliths. According to Czapek (1902) homogentisinic acid is produced in geotropic root tips and phototropic sprout tips, but the mechanism of its action is not clear.

Loeb has shown that phototropism obeys the Bunsen-Roscoe (1862) law. That is to say, the time required to induce a reaction is inversely proportional to the intensity of the light.

The perception of light is perhaps beyond the pale of physical chemistry at present. It should be noted, however, that many of the devices used in photography were first used in the construction of the eyes of animals. Even the "Lumiere" plate for color photography is represented by the retina of turtles and diurnal birds, which is covered with red and orange oil droplets. These are absent in the eyes of nocturnal birds (Winterstein, 1910 iv 577). The rods and cones beneath a red oil drop can be affected by red rays only and those beneath a yellow drop by yellow rays only, whereas those not covered by oil drops would be affected chiefly by the more effective blue rays. In this way a mechanism for color vision is provided, but whether it functions as such has not been determined.

One striking characteristic of the behavior of organisms and parts of organisms is their periodicity. The human body shows a diurnal periodicity that is perhaps most strikingly demonstrated in fluctuations in temperature, being coldest some time near 3 A. M. and warmest about 10 A. M. Each organ has its characteristic periodicity, respiratory center 16 and heart 72 per minute and the motor ganglia 50 per second. It is not always easy to determine what factors determine the period when it is very long. The Atlantic Palolo worm (Eunice fucata) comes out of its hole to liberate the genital segments before dawn on the last quarter of the moon between June 20 and July 25. The light or tide of the last quarter are unnecessary (A. G. Mayer). It is possibly the light of the full moon that prevents it from swarming too soon (some swarmed near full moon this year but another swarm occurred on the last quarter; it was not observed whether the full moon was overcast).

According to Bohn (1914) snails taken far from the seashore maintain a rhythm synchronous with the tides for a considerable period. This rhythm must be due, therefore, to metabolic changes, in the same way that the rhythm of the organs of the human body is maintained. Various physical models have been made showing periodicity, perhaps the most interesting of which are the periodic catalysis mechanisms of Bredig and his pupils. Under certain conditions mercury causes the decomposition of H_2O_2, and this in turn causes a film of oxide to form on the mercury. In these models the film breaks periodically and is reformed. If a crack appears in the film the potential difference between mercury and oxide dissolves the film electrolytically. The surface tension of the mercury differs from that of the oxide, so that surface tension movements as well as catalysis are produced (see Bredig, 1907, B. and Fajans, 1908, B. and Fiske, 1912, B. and Weinmayr, 1903).

Cell Division

Cell division in animal cells seems to be a special form of ameboid motion, and many cells show irregular ameboid movements immediately before division. It is thought by some investigators that the division of the nucleus is necessary before division of the cytoplasm can take place. On the contrary, it was found that the cytoplasm of cells from which the nuclei were removed is capable of division (McClendon, 1908). The unfertilized starfish egg may be caused to divide by immersing it in sea water charged with CO_2, as will be described later. The nuclei (maturation spindles) were removed from starfish eggs and immersed in carbonated sea water, developing imperfect mitotic figures (cytasters) and dividing into spherical masses of protoplasm. In the parthenogenetic frog's egg, to be described later, the cytoplasmic division commences an hour or more before the nuclear division commences, seeming to indicate that the nuclear division is not the direct cause of the cytoplasmic division.

Bütschli, Rhumbler and others considered cell division to be the result of changes in surface tension. The constriction of the cytoplasm follows a band of relatively high surface tension. There seems to be no way of determining whether the surface tension is increased at the equator or reduced at the poles, but the division is often preceded by a rounding up of the cell, which is probably the result of a general increase in surface tension.

That the surface tension at the equator is greater than at the poles of the dividing cell is demonstrated by the superficial movement of the protoplasm. When the tension of a surface is locally altered surface currents pass from the regions of low tension to regions of high tension. Gardiner (1897) observed the superficial granules in certain dividing eggs to pass in the direction from the poles to the equator. The superficial granules in the Arbacia egg become heaped up at the equator during cell division (McClendon, 1910 e), and must have moved in this direction, since they are evenly distributed before division commences. Conklin (1908) observed somewhat similar movements in the eggs of Crepidula.

It is difficult to make a model of cell division because it is difficult to maintain local differences in surface tension long enough for complete division to take place. The following model requires considerable skill to manipulate. A drop composed of one part chloroform and two parts rancid olive oil is immersed in water and prevented from touching the bottom by a layer of concentrated salt solution under it. Two pipettes filled with .1 normal alkali are placed near opposite poles of the drop and the alkali allowed to flow onto these two poles simultaneously. The drop bulges at the poles and constricts in the middle. The reduction of the surface tension at the poles causes surface currents toward the equator, which spread the alkali over the whole surface of the drop. In some instances, however, division is completed before this is accomplished.

Fertilization

Many egg cells do not divide unless they are fertilized by sperm, and it was formerly believed that they are incapable of division unless fertilized.

The process of fertilization may be divided into the following factors and stages: first, the finding of the egg by the sperm; second, the change in the egg responsible for cell division; third, the entrance of the spermatozoon into the egg and fusion with the female pronucleus; and fourth, the transmission of the paternal characters to the egg.

F. Lillie (1913) observed that the sperm of Nereis and Arbacia are attracted by substances given out by the eggs. Since he found that they are attracted by CO_2 and this observation has been confirmed (McClendon, 1914 e), it seems probable that it is the CO_2 given out by the eggs that attracts the sperm, and hence it is not at all specific. Mammalian sperm appear to be attracted by cells of the wall of the uterus.

F. Lillie claims that the sperm cannot unite with the egg unless a substance which he calls fertilizin (a specific agglutinin?) is present to accomplish the union. When the sperm are treated with the agglutinin extracted from the ripe eggs of the same species, they become motionless with their heads adhering together. F. Lillie claims that these agglutinins are specific, but J. Gray (1915) has substituted hydroxyl ions or trivalent cations. Gray says that cerous chloride is as effective as the agglutinin extracted from the egg. He says further that OH ions and trivalent cations not only agglutinate but also activate the sperm, and that H ions, which reduce their activity, do not agglutinate. This inactivation by acids is reversible. F. Lillie had already stated that alkalis cause agglutination and also that acids cause aggregation. Since the attempts of Arrhenius and others to reduce immunochemistry to a physicochemical basis have been so hampered by lack of pure samples of the immune bodies, the discussion of the relation of fertilization to immunochemistry seems out of place at present.

The sperm does not need to penetrate the egg in order to cause cell division. Kupfelweiser, Bataillon (1910) and Brachet observed that the sperm needed merely to scratch the surface of the egg in order to make it segment. The sperm of foreign species usually cannot enter the egg, yet they may cause it to develop. This seems to indicate that the sperm causes a superficial change in the egg. Loeb called this a superficial cytolysis.

R. Lillie observed that some of the red pigment comes out of Arbacia eggs when they are treated with parthenogenic agents, and concluded that fertilization is accompanied by increase in permeability. Lyon and Shackell (1910 b) showed that some of this pigment came out of the eggs on fertilization.

In order to test this conclusion, the electric conductivity of sea urchin eggs immediately before and after fertilization was measured (McClendon, 1910 c, e). The egg is surrounded by a mucous jelly and after fertilization a membrane, the fertilization membrane, appears between the jelly and the egg. Both of these might vitiate the conductivity experiments. It was found that if the jelly is washed from the eggs before they are fertilized, no fertilization membranes appear.

The eggs from which the jelly was washed (with sea water) were placed in the conductivity cell and gently centrifuged. The level to which the eggs came was marked on the cell and the conductivity determined by the Kohlrausch method. The eggs were then transferred to a large quantity of sea water with just enough sperm to fertilize all or at least 80 per cent of the eggs, then washed in fresh sea water and returned to the cell. The eggs were centrifuged down to the same level as before fertilization and the conductivity again determined, which was found to be greatly increased. This experiment was repeated many times with the same result. It was also confirmed by J. Gray (1913 a, b) who observed that about fifteen minutes after fertilization the conductivity began to fall again.

Since the spaces between the eggs were the same during the two determinations and were filled with sea water having the same conductivity in each determination, the change in conductivity is evidently due to the change in permeability of the eggs to ions.

This increase in permeability should decrease the polarization and increase the surface tension, supported by the fact that the eggs round up. The division of the eggs must be due to local changes in surface tension, and is therefore not the direct result of the initial permeability change, but probably an indirect result of it.

After the general increase in permeability, the original semipermeability is partially regained. It is only necessary to assume that the semipermeability is regained first at the poles, thus leaving the equator with the greatest surface tension, in order to account for the division.

The increase in permeability of the egg on fertilization was demonstrated in other ways. It was found that fertilized Arbacia eggs are more easily plasmolyzed by cane sugar solution than are unfertilized eggs, indicating permeability of the fertilized eggs to ions. This may be due, however, to increased permeability to water, observed by Lillie (1916 a).

Lyon and Shackell (1910 b) observed that Arbacia eggs are more permeable to many dyes after fertilization. E. N. Harvey (1911) observed that strong bases penetrate fertilized sea urchin eggs more easily than unfertilized. He seems to consider the alkali, however, as a permeability increasing agent to which the fertilized egg is the more susceptible.

The permeability of the frog's egg increases on fertilization (McClendon, 1914 a, 1915 a). These eggs will live and develop in distilled water. If any substances diffuse out of them, therefore, they may be detected in the water, and the permeability to the contained substances determined. The permeability of the egg to Na, K, Mg, Ca, Cl, SO_4, and CO_3 is doubled by fertilization. Unfortunately it was not possible to measure the increase accurately in less than fifteen minutes in order to determine whether the original increase is partially reversed, as is the case with the sea urchin's egg.

According to Heilbrunn (1915) all initiation of development involves gelation of the substance of the egg. It is not understood how he can distinguish between gelation of the whole and gelation of parts, such as the membrane and the astral rays. It is well known that the astral rays are highly viscous (by centrifuge experiments) and a resistance of the egg to deformation before the appearance of the astral rays might be due to increased viscosity of the membrane.

The formation or separation of a fertilization membrane around the Echinoderm egg has given rise to much discussion. Heilbrunn says it is due to decreased surface tension. Loeb supposed it due to secretion of an osmotically active substance beneath the membrane. Glaser (1914) claims that the egg shrinks during the process which is further evidence of increase in permeability. An apparently similar process occurs in the frog's egg and Bialaszewicz (1908) has shown that the egg shrinks during the process.

Artificial Parthenogenesis

The eggs of some animals develop without the aid of a spermatozoon, a process known as parthenogenesis. Parthenogenetic development may be complete, as in case of the plant lice, or incomplete, as in case of the eggs of hens and silk worms, which if not fertilized, segment but die before development is complete.

Hertwig and Morgan observed that the unfertilized sea urchin's egg may be caused to segment by treatment with certain solutions. Loeb continued these experiments with the object of obtaining complete development, calling it artificial parthenogenesis. Delage succeeded in raising several such sea urchin's beyond the larval stage. Bataillon reared several parthenogenetic frogs, and Loeb confirmed his experiment.

Loeb observed that cytolytic agents cause membrane formation on sea urchins' eggs, considering this due to a superficial cytolysis, also considering the development of the egg to be due to two factors, first, membrane formation, and second, a corrective factor which was not clearly defined.

R. Lillie supposed the development of the eggs to be due to increase in permeability. It was found that the electric conductivity of the sea urchin's egg increased when it was made parthenogenetic to the same degree that it did when it was fertilized (McClendon 1910 c, e). The eggs were made parthenogenetic by treatment for a few seconds with a dilute solution of acetic acid in sea water, then being thoroughly washed in sea water. This increase in conductivity must be interpreted as an increase in permeability of the eggs to ions.

The permeability of the frog's egg increases when it is made parthenogenetic (McClendon, 1914 a). It had been observed that the frog's egg will commence segmentation if a 110 volt alternating current is passed through the water containing the eggs from electrodes about two inches apart, for about one second (McClendon, 1911 b). This stimulation increases the permeability of the eggs to the same extent that fertilization does.

Since both fertilization and artificial stimulation act in the same way in increasing the permeability of the eggs, we have cumulative evidence to indicate that the increase in permeability is essential to segmentation.

The kinds of stimuli capable of inducing artificial parthenogenesis are very numerous. Heilbrunn (1913) claims that they all decrease the surface tension of the egg. It seems more probable, however, that the surface tension is increased, since the egg becomes spherical. It was found possible to cause the eggs of Arbacia punctulata to segment by treatment with any one of the following: hypotonic solutions, isotonic solutions of acids (especially fatty acids), alkalis or neutral salts, hypertonic solutions, mechanical shocks, thermal changes, electric currents, KCN, lack of oxygen, or anesthetics (McClendon, 1909 b). To these may be added in order to complete the list of parthenogenic agents, soap, bile salts, alkaloids, glucosides, blood sera, ultraviolet rays, and simply pricking with a needle. We thus have a list about as large as the list of stimuli for nerves. In what way all these agents increase the permeability is not known. The discussion of this question should be under the general topic of stimulation.

The question as to whether the fertilization membrane is present before fertilization has been much discussed. Harvey (1910 a) says that it is not present, whereas Kite takes the opposite view. It has been supposed to be formed by the contact of the transparent gel covering the egg with a secretion poured out by the egg at fertilization, since it is absent from eggs that have been washed a long time, during which the gel is removed (McClendon, 1911 a). Harvey supposes this washing injures the egg. The evidences for a secretion are numerous. The frog's egg, and according to Glaser (1914 b) the Echinoderm egg, gets smaller during this secretion. By the formation of this membrane it is merely supposed that a denser or more viscous layer is formed out of material already present. It has been shown by microdissection that the surface of the unfertilized egg is not as viscous as the fertilization membrane (McClendon, 1914 e). Heilbrunn (1915 a) supposes that the viscosity of the whole egg is increased by fertilization, but his proof that this increase in viscosity is not confined to the fertilization membrane is not clear. Loeb supposed the elevation of the membrane to be caused by the secretion of an osmotically active substance of high molecular weight behind it. Kite says the membrane swells and that we see the outer surface of it. It is probably pushed out by the passage inward of water attracted by a colloid, but whether this is due to the osmotic pressure or swelling pressure of the colloid seems less important, as it is not certain that these two phenomena are different.

Many attempts to cause the eggs to segment with sperm extracts have been tried, but positive results have been attributed to impurities or lack of isotonicity (M. Morse, 1912 a). Since the sperm contains lecithin and therefore fatty acid, and since fatty acids and many other organic substances cause membrane elevation and segmentation, it should be possible to cause segmentation with sperm extract. Robertson (1912) washed sperm in H_2O, precipitated the extract with $BaCl_2$, redissolved it in HCl and reprecipitated it with acetone. The resulting "oocytin" caused membrane elevation.

CHAPTER XIV

MUSCULAR CONTRACTION, OXIDATION AND HEAT AND LIGHT PRODUCTION

The muscle was at one time considered to be a machine in which a difference in temperature produced by oxidation is converted into mechanical energy, but this hypothesis does not harmonize at all with the facts. A. V. Hill (1913 a) has shown that when the muscle performs an isometric contraction in an atmosphere of nitrogen the heat produced is about half as great as when oxygen is present, and develops simultaneously with the contraction. Evidently this is a case, at least in part, of the transformation of mechanical work into heat. If oxygen is present, the heat produced is much greater, but nearly half of it is produced after the contraction is completed, evidently having nothing to do with the contraction.

Striated Muscle

The muscle may work for a considerable period without oxygen, or with the oxidations suppressed by HCN. Weizsäcker (1912) reduced the respiration of frog's heart to 36 per cent of the normal for thirty minutes without reducing the work performed. By larger doses of HCN he completely prevented the consumption of oxygen, finding the work to be only reduced to about 70 per cent of the normal. This proves conclusively that the muscle is not a heat machine.

Hill measured the heat and work produced by a muscle with a relatively low tension and stimulus, and found the work to equal 90-100 per cent of the equivalent of the heat produced during contraction. The heat produced in the relaxation period was 80 per cent of that produced during contraction. Therefore the efficiency of the muscle is more than 50 per cent.

Engelmann (1893) showed that a catgut stretched in water would contract on addition of acid. Stretching of the catgut while swollen makes it anisotropic and if dried in this condition it will remain so until swollen again. The muscle fiber is anisotropic. Meigs (1908 b) has shown that there are no isotropic discs, but merely regions of less density or anisotropicity. Engelmann showed that the catgut would not contract on swelling unless anisotropic.

It has long been known that sarcolactic acid is produced by an active muscle, and increases in proportion to fatigue in a muscle in a nitrogen atmosphere. Fletcher and Hopkins (1907) have recently reinvestigated this question. If oxygen is admitted to the fatigued muscle in nitrogen, the lactic acid disappears, and CO_2 and heat are produced. Some suppose the lactic acid to be the cause of the contraction. Death rigor has been compared to a long continued contraction, and is accompanied by lactic acid production. Winterstein (1897) has shown that an oxygen atmosphere may prevent the production of acid and likewise the death rigor in a dead muscle. Death rigor is apparently caused by a certain low concentration of acid, and when the acid increases above this the rigor disappears (Von Fürth and Lenk, 1911). This is in harmony with the observation of Spiro and others that low concentrations of acid cause swelling of hydrophile colloid gels, whereas higher concentrations do not. Von Fürth and Lenk (1911) have shown that the muscle during death rigor and immediately before it, has a great power of absorbing water, but losing this power and even shrinking a little, due to loss of water, after the passing of the rigor. The shrinking is apparently due to the excess of acid. At a low acid concentration the muscle proteins swell, shrinking at a higher concentration.

McDougal (1908), Meigs (1908 a) and others have revived Engelmann's swelling theory of muscular contraction, with the substitution of osmotic pressure, as illustrated by the following model. A rubber tube has a series of close fitting metal rings slipped over it so that the distance between them is less than the diameter of the tube. The tube is closed at one end and water is pumped into the other. As the volume of the contained water is thus increased the length of the tube is decreased. The metal rings are supposed to take over the function of the Krauze's membranes of the muscle fiber. Later Meigs (1910) returned to Engelmann's idea that colloidal swelling is the cause of shortening.

Bernstein (1901) assumed that increase in surface tension caused the contraction of muscle. This hypothesis has the advantage that it relates the contraction to the electrical phenomena. As we previously noted, depolarization causes increase in surface tension. An increase in surface tension of the whole fiber, or even of the myofibrils, would have to be very great in order to account for the force of contraction of the muscle. Bernstein supposed the contractile elements to be minute ellipsoids, whose elasticity tended to maintain the elongated shape, whereas an increase of surface tension would make them more spherical.

Mathematical data may decide between these two theories. Bernstein (1908) points out that the temperature coefficient of muscular contraction as well as of surface tension is negative, whereas that of swelling of colloids is positive. He further shows (1915) that violin strings, such as used by Engelmann, are twisted, and if untwisted they do not contract on swelling.

R. Lillie supposes the surface tension increase to affect the colloidal particles in the muscle and cause their aggregation. This aggregation of colloidal particles is what is often expressed by the word coagulation. The experiments of von Fürth show that death rigor, at least, is not a coagulation but the reverse, a colloidal swelling.

Smooth Muscle

When smooth muscle contracts it remains so without fatigue and needs another stimulus (inhibitory nerve) to relax it. This is called the catch mechanism, and enables it to hold up a greater weight than an equal volume of striated muscle and yet without expenditure of energy. These facts together with the statement by Meigs (1912 c) that smooth muscle has no semipermeable membranes prevent the extension to it of the theory of muscle contraction just described.

Oxidation

In the enthusiasm following the discovery of enzymes, animal and plant oxidations were thought by some investigators to be explained by merely assuming that they were brought about by oxidases. With the search for oxidases many have been found and some of them seem to be specific. The fact, however, that no series of oxidases with which the complete oxidation of the ordinary foodstuffs can be accomplished have yet been isolated from the organized cell or cell fragment, places the question of oxidations in a separate field of research from enzymes in general. Harden and McLean (1911) found that respiration did not occur in the press juice of muscle and some other tissues. Batelli and Stern (1909) confirmed this observation but claim that respiration occurs in the press juice of liver. Warburg (1913) showed that most of the oxidation in liver juice is associated with granules. Whereas the oxidation of the liver juice is 20 per cent of that of the intact liver or liver cells, the oxidation of the juice after having passed a Berkefeld filter is only 4 per cent of that of the liver. As shown by Bechhold, such treatment may not remove all of the colloidal particles. Warburg found that the granular mass of ground sea urchin eggs absorbs oxygen but does not give out CO_2. Warburg and Meyerhof (1914) found that the lecithin extracted from the egg when mixed with a ferric salt the equivalent of the iron in the egg, absorbed as much oxygen as the ground egg. This is probably due to the oxidation of unsaturated fatty acid radicals in the lecithin, and is accelerated by H ions. A similar reaction takes place in linseed oil and is accelerated by various metallic and organic oxides or peroxides. Warburg (1912 a) ground young erythrocytes (with rapid respiration) in a special ball mill (Macfadyen and Rowland) and the oxidation was reduced to zero.

Much of the earlier theoretical work on oxidations is based on the assumption that O_2 is relatively inert and that nascent oxygen or some active oxide or peroxide is necessary to affect oxidation of difficultly oxidizable substances. This follows from the fact that oxidation consists in the addition of a positive charge or valence to the molecule. The molecule thus charged may combine with OH, or if the charge is double it may combine with one atom of oxygen. What is meant by nascent oxygen is not clear. Perhaps some substance splits out O'' from O_2, thus leaving $O^{\cdot\cdot}$ which can exist only in a nascent state because it so easily parts with its positive charges, and in doing so oxidizes the substance receiving them.

Various unsaturated compounds have been supposed to aid in oxidations by combining with one atom of the oxygen molecule thus leaving the other free to attack another substance that is not so easily oxidized. According to Bach and Chodat (1904) an oxidase is a mixture of two enzymes, oxygenase and peroxidase. The oxygenase combines with O_2 to form a peroxide which is attacked by the peroxidase, with the liberation of active oxygen that in turn attacks the difficultly oxidizable substance present. H_2O_2 can be substituted for these peroxides. One criticism of the study of the oxidases is that relatively easily oxidizable substances have been selected with which to test their activity. It would be interesting to have a list of organic substances arranged in order of oxidizability, as well as a record of the oxidizing power of cell constituents of recognized purity if not known constitution. The author observed that oxyhemoglobin (recrystallized five times) as well as metahemoglobin (recrystallized seven times) would accelerate the oxidation of a-napthol, aloin and p-phenylene diamine and an alkaline solution of a-napthol + p-phenylene diamine, whereas hematin would not accelerate the oxidation of these substances without the addition of H_2O_2.

Since H_2O_2 or some other peroxide is necessary for the activation of the peroxidases of the cell, all cells contain catalase which decomposes H_2O_2 with enormous rapidity, with the liberation of inactive oxygen. It has been suggested that catalase protects the protoplasm against oxidation. But how is the oxidation regulated so as to occur at the right place at the right time? The answer to this seems to lie in cell structure, which will now be considered.

It was stated in the chapters on the bioelectric phenomena and muscular contraction that when a muscle contracts dextrose disappears, the permeability of the cells is increased, lactic acid appears between them, if oxygen is admitted the lactic acid disappears, and CO_2 and heat are produced. The simplest explanation of this chain of phenomena is that in the muscle fiber lactic acid is produced until the reaction is stopped by the accumulation of this end product. This would make the reaction of the protoplasm slightly acid, but it need not even reach the isoelectric point of the proteins, so that the proteins may continue to be electronegative. That the cell sap of certain plant cells is acid is shown by their red color, and indicators swallowed by protozoa have shown an acid reaction of the vacuole contents. But it is impossible to determine the reaction of the protoplasm itself, except to say that it is less acid than the isoelectric point of the proteins or lipoproteins of which it is formed. As soon as the lactic acid reaches the outer surface of the plasma membrane it is burned, provided O_2 is present. As will be shown later, the oxidase concerned is probably associated with the plasma membrane, possibly adsorbed to its outer surface. Anesthetics might suppress oxidation in two ways, by preventing the permeability increase, or by coating the oxidase with a layer preventing the access of the oxidizable substance.

The evidence that such a process might take place in other cells is found in the fact that the oxidation of the sea urchin's egg increases sevenfold when its permeability increases, and Warburg (1911 b) has shown that the oxidation of erythrocytes is increased on laking by freezing and thawing (provided there is not too much serum or salt solution present). Warburg gives as evidence of surface action the fact that alkaline sea water or pure NaCl solution increases the oxidation of unfertilized sea urchin eggs sevenfold whereas they do not penetrate the protoplasm. He takes experiments of Overton as evidence that NaCl does not penetrate, and as evidence that NaOH does not penetrate the fact that this concentration of NaOH does not turn eggs yellow that have been stained with neutral red, whereas NH_4OH of less alkalinity does.

Colorless substances may be oxidized by cells to colored compounds that appear as granules in the cell interiors. This does not prove that oxidation occurred in the exact spot in which the granules are seen. Certain granules in cells absorb or adsorb and concentrate dyes so that they may remove all of the dye from their surroundings. Even though an oxidation product is said to be insoluble, solubility is a relative term, and the diffusion of an extremely dilute solution of a colored substance cannot be detected with the microscope because this instrument does not make the color appear more intense than it actually is. But it does not seem reasonable that all of the oxidation takes place on the cell surface. Warburg (1911 b) has shown that minute quantities of NH_3, insufficient to change the reaction of the serum to indicators, may increase the oxidation of erythrocytes, whereas NaOH is ineffective. Since NH_3 penetrates so much more easily than fixed alkalis, this indicates that NH_3 affects oxidation in the interior, or else the action is on the plasma membrane.

Warburg found that anesthetics reduced the oxidation of erythrocytes according to their anesthetic power. He illustrated it by the following model (1914): Blood charcoal causes the oxidation of oxalic acid adsorbed to its surface to CO_2 and H_2O. This charcoal, even after washing with HCl, contains iron which may be the real catalyzer. The catalytic action of the charcoal varies with its power of adsorbing oxalic acid. If an anesthetic is added to the solution of oxalic acid the oxidation is reduced. The more easily the anesthetic is adsorbed the greater is its inhibitory action. The explanation of this action is that the anesthetic is more strongly adsorbed than the oxalic acid, which it displaces from the surface of the charcoal.

McClendon and Mitchell (1912) showed that those solutions that were most effective in increasing the permeability and causing the segmentation of unfertilized sea urchin eggs, are also most effective in increasing the oxidation. Warburg (1910 a) combats the idea that the segmentation is the cause of the oxidation. He finds that phenyl urethane may stop the segmentation without reducing the oxidation to a significant degree. But the segmentation and increased oxidation might be due to increased permeability without the two running parallel. Let us suppose that oxidation is proportional to permeability. A too great increase in permeability will prevent segmentation, but it might greatly increase oxidation.

Whereas Warburg is forced to admit some connection between the plasma membrane and oxidation, he claims this does not hold true for the unfertilized sea urchin egg, whose oxidation is not decreased by grinding with sand. But this fact is capable of another interpretation. It may be a mere coincidence that the absorption of oxygen by the intact and the ground egg is the same, since the production of CO_2 ceases on grinding. The first impact of the sand may cause the eggs to begin development, since they may be caused to segment by shaking in a vial. In this condition they are the same as fertilized eggs, with enormously increased oxidation. This view is supported by the fact that Warburg found the oxidation of unfertilized eggs burst in distilled water to be temporarily increased. They may be caused to develop by a short treatment with distilled water or longer treatment with hypotonic solution. After the egg is ground with sand the oxidation falls to the same level as a fertilized egg ground with sand, and in neither case is CO_2 given out. The CO_2 output of the unfertilized egg is totally changed (abolished) by grinding and the fact that the O_2 absorption is the same at a certain time after grinding may be a mere coincidence.

Warburg and Wiesel (1912) have attempted to show the similarity between the oxidizing power of cells and enzyme action, in the reduction of oxidation and fermentation of yeast to the same degree by anesthetics. The activity of the zymase in yeast press juice is but a small fraction of the activity of the yeast, but the oxidation has been reduced to nil. There is a slight oxidation in the filtered liver press juice, however, and this may be compared to the zymase in yeast juice. The significance of cell structure, however, is shown by the fact that the oxidation of various cells is reduced to about one-third by killing the acetone, which leaves part of the structure intact.

The results of Batelli and Stern (1914) also indicate a relation between cell structure and oxidation, but they probably interpret it differently. They divide respiration into chief or rapid and accessory or lasting respiration. This accessory respiration seems to be due to cell fragments, dead cells, cell granules (and bacteria?) and only a small percentage of cell juice proper. They also studied the oxidizing power of ground tissues on various substances and conclude that the citric acid oxidase ("oxidone") is destroyed with cell structure, but the others are not. They attempted to get more oxidases in the extract by grinding the tissue finer, using a Borrel mill with revolving knives, but this treatment merely reduced the oxidation of the cell fragments, without increasing the oxidation of the extract.

Warburg observed that the oxidations that take place in cell extracts may be reduced by anesthetics, but the concentration of anesthetic required is many times that required to reduce the oxidation of the intact cell a corresponding amount. He explained this as due to the fact that the oxidase in the cell is adsorbed to a surface, the anesthetic adsorbed to the same surface, and its concentration increased above that in the interior of the solution. Meyerhof (1914 a) attempted to make a model to show this difference. He found that the activity of an invertase solution is not altered by adsorption to colloidal ferric hydrate (dyalized iron). If the same concentration of anesthetic is added to the invertase solution and the invertase iron suspension, the activity of the latter is reduced more than the former, but the difference is very small, not nearly so great as between the invertase inside and outside the cell.

The question as to whether the cells of the body would use more oxygen if they were more liberally supplied with it is very important. Paul Bert (1878) found that animals died in oxygen under pressure, but this phenomenon has never been thoroughly analyzed. Anaerobes and facultative anaerobes may live indefinitely and many other organisms may live for a time without oxygen. Infusoria may live from five to twenty days without oxygen (Putter, 1911). The development of Fundulus eggs may be suspended by withholding oxygen and commence again when it is readmitted. Henze (1910) found that sea anemones use more oxygen the greater its concentration in the sea water. When protein is eaten the mammal uses more oxygen than when a carbohydrate of the same calorific value is eaten. One explanation of this might be that the protein gives rise to acid substances in the blood which stimulate the respiratory center and cause more oxygen to be brought to the tissues.

Heat Production

The heat produced by burning fats and carbohydrates in the body is the same as that obtained in burning them in the bomb calorimeter, 3.74 Cal. per gram of monosaccharide, 3.95 for disaccharide and about 9.5 for ordinary animal or vegetable fat. But the heat produced by protein is 5.7 in the calorimeter and only 4.5 in the body. The difference is that the protein is not completely burned in the body, being excreted as urea, uric acid, NH_3 and other incompletely oxidized products.

While heat production and loss in man is so regulated from the heat center in the brain that the temperature of the body, to within an inch of the surface, seldom varies more than a degree during health, this is not at all the case with the lower organisms. The temperature of small marine animals is never more than .7° above that of the medium. The body of an isolated insect may be 4° above that of the air, whereas the temperature in the center of a swarm of bees may be 17° above that of the air.

Light Production

Light is produced by bacteria, fungi, protozoa, medusae, ctenophores, molluscs, fish, crustacea and insects, and the eggs and larvae of photogenic animals. The sea on a favorable night reveals a host of wonderful luminous creatures. The effect of myriads of luminous Dinoflagellates in the Pacific is very striking. The water is reddened by day and turned to a sea of fire at night by these protozoa. Spray, the crests of waves and the outlines of fish and bathers are illuminated. The species is Gonyaulax polyedra.

In all organisms which produce light intermittently, the production of light follows stimulation with all classes of stimuli. If the production of light is always due to oxidation, it forms a convenient index of the control of oxidation by the organism or cell. When the photogenic organism is crushed, the light is emitted at a rapid rate for a short time. An analogy to this luminescence of the dead organism is seen in "chemiluminescence" which will now be described.

It was shown by Callaud and Pelletier that wax, fatty oils, sugar and alkaloids, emit light on being warmed. Radziszewski (1883) showed that these substances even if kept in the dark, and not previously exposed to light, emit light on oxidation with alkaline solutions containing O_2. Besides glucose, gallic acid, etherial oils, cholesterin and lophin, he found that unsaturated fatty acids and compounds containing them may emit light. His list includes oleic, elaidic and ricinoleic acids and their fats and soaps and lecithin, protagon and cetyl alcohol wax.

It has been pretty clearly established that the production of light by animals and bacteria is dependent on oxidation. Recently E. N. Harvey (1914) has dried the photogenic organs of the firefly in vacuo and found that it could be kept in this condition indefinitely and at any time caused to emit light by the addition of water containing O_2. Harvey (1916) obtained from

Japanese animals luminous secretions and extracts that are free from particles. According to Raphael Dubois (1913), these secretions contain "luciferase" which can be replaced by potassium permanganate and "luciferin," of unknown chemical nature. Harvey (1916) finds that various oxidases may be substituted for luciferase.

Coblentz (1912) photographed the spectrum of the light emitted by two species of firefly of the genus Photinus. The light contains only from yellowish green to orange rays. It is a cold light and is also lacking in rays of short wave length. He found the temperature of the luminous organ to be only a fraction of a degree above that of the same organ when not emitting light. It is probable but not certain that this heat is produced by the oxidation of the photogenic substance, as the admission of oxygen to the organ would naturally result in cell respiration.

CHAPTER XV

BLOOD AND OTHER CELL MEDIA

The coagulation of blood is nothing more than a gel formation of a protein hydrosol, the gel being called fibrin and the same substance in colloidal solution, fibrinogen. According to Howell (1914) the fibrin clot is a mass of acicular crystals. This coagulation leads to the distinction of plasma, the medium in which the blood corpuscles float, and serum, the same minus the fibrinogen.

As was previously stated, the permeability of the blood corpuscles seems to be nearly the same as the permeability of plant cells. Overton claimed plant cells to be impermeable to salts. The same seems to be true of blood corpuscles for otherwise how is the difference between the salts of the plasma and serum maintained since Höber has shown that the ions are not bound but free to move within the corpuscles. Abderhalden gives the following data of the salts of corpuscles and plasma in parts per hundred:

		Horse	Pig	Rabbit	Ox	Sheep	Goat	Dog	Cat
Na in.	serum	.4396	.4251	.4442	.4312	.4294	.4326	.4278	.4439
	cells	——	——	——	.2232	.2257	.2174	.2839	.2705
Cl in..	serum	.0259	.0270	.0259	.0255	.0255	.0246	.0245	.0262
	cells	.4130	.4957	.5229	.0722	.0741	.0679	.0273	.0258
K in..	serum	.3690	.3627	.3883	.3690	.3704	.3691	.4080	.4170
	cells	.1205	.1475	.1236	.1813	.1725	.1480	.1327	.1048
H_2PO_4	serum	.0076	.0052	.0064	.0085	.0085	.0070	.0081	.0071
	cells	.1687	.1653	.1733	.0350	.0365	.0279	.1256	.1186

Notwithstanding this evidence Hamburger claims that the corpuscles are freely permeable.

The main difference noted between the permeability of plant cells and blood corpuscles was in case of urea, to which the plant cell is said to be impermeable, whereas some blood corpuscles, at least, allow it to pass through. It would be interesting to test the permeability of the erythrocytes of elesmobranchs (sharks and rays) to urea, since its concentration in the blood of these fish is about 5 per cent, whereas it exists only in very small quantities in the blood of other animals.

The osmotic pressure of the blood of warm blooded animals is about eight atmospheres, about as constant as the body temperature. As we descend in the animal kingdom the osmotic pressure of the blood is more and more influenced by the water the animal drinks or lives in, reptiles, amphibians, bony fish, and all air and fresh water animals, being more or less independent of the medium. The osmotic pressure of the blood of all other marine animals is the same as that of the sea in which they live. The following table gives the freezing point lowering of the blood (or of some fluid approximately isotonic with the blood) of a number of animals:

MAMMALS	Δ degrees		
Man, serum	.56	(.48-.62)	(In cardiac insufficiency up to 1.099)
Ox	.55	(.53-.66)	
Horse	.564		
Pig	.615		
Rabbit	.592		
Dog	.571	(.56-.62)	
Cat	.638		
Sheep	.619		
Whales, blood	.65-.7		
BIRDS			
Fowl	.6-.64		
Goose	.55		
REPTILES			
Caretta, caretta, marine	.61-.69		
Colpochelys kempi, “	.70		
Chelonia mydas, “	.675		
Chelonia caouana, “	.602		
Emys europaea, fresh water	.474	(.46-.65)	
Pseudemys elegans	.48		
AMPHIBIA			
Rana esculenta	.465-.56		
Bufo viridis	.76		
Salamandra maculata	.479		
TELEOSTS (bony fishes)			
Charax puntazzo, marine	1.04		
Cherna gigas, “	1.035		
Crenilabrus parvo, “	.75		
Box salpa, “	.85		
Pleuronectes platessa, “	.65-.85		
Amia calva, land locked	.508		
Anguilla vulgaris, fresh water	.58-.69		
Barbus fluviatilis, “	.48-.56		
Catostomus teres, “	.51-.52		
Leuciscus dobula, “	.45		
Perca, Cyprinus, Tinca, Esox, “	.512		
a fluviatilis, “	.5-.51		
o trutta and alpinus, “	.62		

GANOIDS	
Acipenser sturio	.76
Lepidosteus osseous	.49-.52
Polyodon spathula	.486-.50
INVERTEBRATES (excepting marine) (blood + body juices)	
Anodonta cygnea	.2
Astacus fluviatilis	.8
Dytiscus marginalis	.57
Hirudo medicinalis	.4-.43
Dragon flies	.65

The osmotic pressure of the blood plasma must be imitated in any solution used to wash the corpuscles, as otherwise cytolysis (hemolysis) may result. If NaCl solutions are desired, .95 per cent is used for mammals, reptiles and birds and .65 per cent for frogs. A 1 per cent $CaCl_2$ (anhydrous) solution, or .74 per cent KCl, or .75 per cent NaCl has a $\Delta = .465$. In order to reduce the toxicity of the NaCl, the solutions described at the end of the chapter are used. If cane sugar is used where a non-electrolyte is desired, about ten times as much is weighed out as in case of NaCl for an equal volume of solution (see Fig. 30). Sugar solutions are slightly toxic, probably on account of the H ions the sugar dissociates. An absolute non-electrolyte has not been found. It is possible that a small proportion of urea added to the sugar solution might reduce the H ion concentration sufficiently.

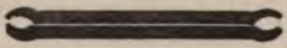


FIG. 29. Viscosimeter for a drop of blood. A little hirudin or oxalate is placed in one bulb, which is filled with blood. The tube is grasped with forceps and inverted. The viscosity is proportional to the time required for the blood to leave the bulb.

The blood in passing through the capillaries of the body undergoes many changes, not all of which are well understood. The effect of CO_2 on the blood is more complicated than was at first supposed. When CO_2 is bubbled through blood the H ion concentration is increased in the plasma and the OH ion concentration reduced, but, contrary to what might be expected, the titratable alkalinity is increased. This is partly explained by the fact that the Cl ions are reduced by the substitution of carbonates for chlorides. Koeppe (1897) put forward the following hypothesis: The CO_2 enters the corpuscles and combines with the

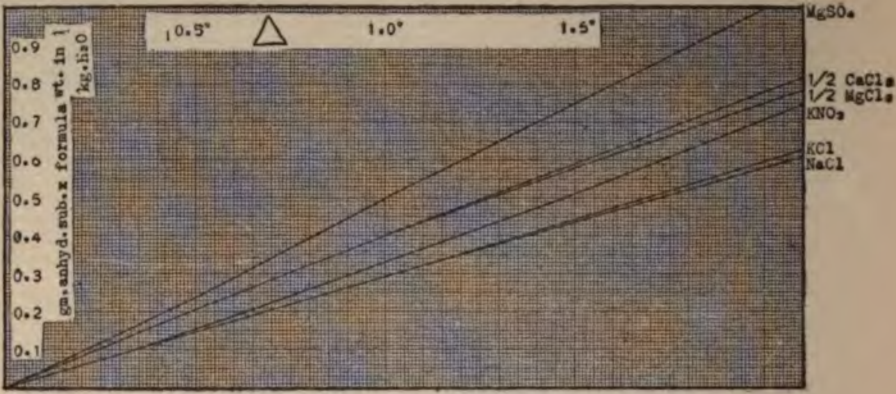


FIG. 30. Freezing point lowering of weight normal solutions. The scale is too small to show the increased ionization at very great dilution. The curve for $MgSO_4$ approximates that for cane sugar and dextrose and is near that for 1/2 K_2SO_4. The curve for KCl may be used for KOH, NaOH and $NaNO_3$. The curve for NaCl approximates that for NH_4Cl and is near that for Na_2SO_4 for the higher concentrations.

proteids. This combination dissociates to a greater extent than CO_2 and the excess of HCO_3 ions so produced diffuse into the plasma, leaving the interior of the corpuscle electropositive. The Cl ions of the plasma are attracted by this electropositive charge and enter the corpuscle. Only a small part of the CO_2 added takes part in this exchange, however, and a larger proportion of it is stored in the corpuscles than in the plasma. This follows from von Limbeck's observation that the osmotic pressure of the corpuscles increases more than that of the plasma, causing the corpuscles to swell, due perhaps to the fact that alkali within the corpuscles binds the CO_2. This alkali is supposed by some investigators to be ordinarily combined with protein, but in the presence of an excess of CO_2, joins the latter.

This experiment shows that, at least in the presence of an excess of CO_2, the erythrocytes are permeable to Cl ions. Hamburger and van Lier (1902) have shown by the same method that the erythrocytes are permeable to SO_4 and NO_3 ions.

Henderson and Spiro (1908) made a model of this process. A beaker of Na_2CO_3 solution represented the plasma, a parchment paper tube filled with Na_2CO_3 + protein representing a corpuscle. CO_2 is bubbled through, the titratable alkalinity of the pure Na_2CO_3 solution is found to be increased because Na-Protein is changed to H-Protein + $NaHCO_3$ and the latter diffuses into the pure solution. As is readily seen, the analogy is not exact.

It is possible that the plasma membrane of the erythrocyte is different from that of other cells. The stroma of these cells contain a large percentage of lecithin and cholesterin. Pascucci (1907) made membranes of lecithin and cholesterin that could be laked with the same reagents that lake corpuscles. These membranes were more easily laked the greater the percentage of lecithin, the same being true of corpuscles. Perhaps some laking agents combine with lecithin. Berczeller (1914) found that the surface tension of alkaline solutions of lecithin is increased by the addition of chloroform or ether. The relation of lecithin and cholesterin in the serum to certain hemolysins has been much studied, lecithin favoring and cholesterin inhibiting hemolysis. According to von Dungern and Coca (1908 a, b, c) the hemolysin in snake venom is a lipase that splits off fatty acid from the lecithin, and the latter hemolyzes the corpuscles. Iscovesco (1910 c) claims that cholesterin inhibits the action of lecithin by combining with it. Lecithin reduces the surface tension of water, but on addition of cholesterin it rises again. He claims that cholesterin acts on soap in the same manner.

Though anesthetics in high concentration are hemolytic, Traube (1908 a) found that small doses inhibit hemolysis. He claims that hemoglobin, itself, when in the serum, is hemolytic, but his specimen of hemoglobin was apparently impure.

The relative volume of corpuscles and plasma may be determined with the hematocrit (Hedin) or calculated from the conductivity (Stewart) volume of corpuscles

$$= 92 \frac{\text{conductivity of blood}}{\text{conductivity of serum}} + 13.$$

The hematocrit was used by Hamburger to find the osmotic pressure on the assumption that the corpuscles are impermeable, although, in a series of later papers, he claims that they are freely permeable.

The viscosity of the blood is determined by the total volume of the corpuscles and to a less extent by the proteins of the plasma. The normal viscosity is 4.05-6.8, is greater in man than in woman, and increases after feeding and after muscular work. In cardiac insufficiency it may reach 8.1 and in polycythemia 14.4-23.8. It is also increased by stasis. A convenient viscosimeter for blood is shown in Fig. 29. (It may be obtained from A. S. Jones, Princeton, N. J.)

The fact that the viscosity of the blood is determined chiefly by the total volume of the corpuscles suggests a method of determining the corpuscular volume. Such measurements must be made at nearly the same temperature, to be comparable. The viscosimeter is standardized with H_2O at the temperature at which the blood is investigated, but the absolute viscosity of water is 27 per cent less at 37° than at 17° whereas this ratio is not the same for blood (blood is said to be 16 per cent less viscous at 37° than at 17° but it is not clear that the absolute viscosity is meant). According to Hess the hemoglobin may be calculated as follows: $\frac{\text{hemoglobin}}{\text{viscosity}} = 19$, except in diseases in which the hemoglobin content of the corpuscles or the plasma proteins or the proportion of leucocytes is changed.

The normal refractive index of the serum of man is 1.345-1.35. According to Pearl (1914) the refractive index of the serum of the fowl is 1.34537 and of the guinea hen 1.34184.

It is usual to consider the solvent power of the blood plasma as being nearly the same as that of water, but this is not always the case. Uric acid is practically insoluble in water, the concentration of its saturated solution at 18° being .00015 mol, the molecular weight being 168.2. Bechhold and Ziegler (1910) found that a liter of serum would dissolve more than half a gram of uric acid, at 37°, showing that its solubility in serum is enormously greater than in water. The reason for this difference is not self evident. Uric acid is a weak acid, in saturated solution in water being only 9.5 per cent dissociated. Although theoretically a dibasic acid it acts like a monobasic acid, as only one H ion is dissociated ordinarily. The primary sodium salt is more soluble than the free acid in water because it is strongly dissociated. According to Gudzent (1909 a) there are two isomeres, a saturated solution at 37° of the laktam form being .01 mol, and of the laktim form being .0068 mol. The solubility is greatly reduced by the addition of Na salts, which accounts for the fact that it is more than thirty times as soluble in water as in physiological salt solution. The primary lithium salt is much more soluble. This has led to the practice of giving lithia water to gouty patients, but it is impossible to decrease the concentration of $Na^{\cdot}$ in the blood to the extent that the solubility of uric acid would be noticeably increased.

Since uric acid, when added to serum, becomes primary sodium urate, and the solubility of this is very low, it seems strange that the solubility of uric acid in serum should be relatively higher than the urate. According to Schade and Boden (1913), however, the uric acid forms a colloidal solution in serum, and in water, also, under certain conditions. If uric acid is dissolved in water by means of heat and the addition of minute quantities of alkali, a 2 per cent solution may finally be obtained. By the addition of neutral salts or by cooling, the solution may be transformed into a transparent gel, which after a greater or less time deposits crystals of uric acid. It is probable that the colloidal solution in serum is protected from crystallization by the other colloids present (protective colloids).

The blood plasma is very slightly alkaline, this alkalinity being maintained by the bicarbonates (and only to slight extent by phosphates) it contains. Höber (1903 b) was the first to measure the H ion concentration of the blood. The later work on this subject has been largely concerned with the perfection of the technique. It has been shown that the PH of the blood is hardly affected by the changes in temperature to which it is ordinarily subjected (McClendon and Magoon, 1916) and, therefore, the determinations of the PH of the blood at different temperatures by different observers would be comparable, provided the proper precautions were taken to prevent loss of CO_2. The technique was described in Chapter IV, 7.5 taken as about the average PH of human blood. The PH of the blood of different individuals is so constant as to come almost within the limit of error of measurement. Hasselbalch and Gammeltoft found the PH of the blood of a number of supposedly normal individuals to vary from 7.4 to 7.49. Michaelis (1914) found the PH of normal human blood, dropped through the air into a relatively large volume of CO_2-free salt solution, to vary from 7.49 to 7.64, but these high (alkaline) values are probably due to faulty technique. Winterstein (1911) showed that if new born pups are perfused with CO_2-free saline, the respiratory movements are not induced, but if a trace of HCl is added to the perfusing fluid, the respiration commences. It was shown by Hasselbalch and Lundsgaard (1912) that the respiratory center in anesthetized dogs is controlled by the H ion concentration of the blood. Milroy (1914) obtained similar results on dogs and cats but claimed that the threshold of stimulation of the respiratory center by H ions is lowered by lack of oxygen. This apparent result of his experiments may be explained away in the following manner: We may suppose that the respiratory center is affected not by the reaction of the blood directly, but by its own reaction, which is in a dynamic equilibrium with that of the blood (by diffusion). During lack of oxygen acid substances produced in the respiratory center fail to be removed by oxidation, and hence the H ion concentration increases although that of the blood remains constant.

In the same way the lowering of the buffer value of the blood in acidosis may affect the respiratory center. The lack of bicarbonate in the blood coming to the respiratory center decreases its power of neutralizing the CO_2 produced by the center, and increased breathing may result. This may cause a decrease in the H ion concentration of the blood and decreased breathing. In this way irregular or labored breathing may arise, the total lung ventilation being increased owing to the low CO_2 tension of the blood and alveolar air. That is to say, a given volume of expired air would carry a subnormal amount of CO_2, and, hence, the lung ventilation must be increased in order to remove the CO_2 from the body at the normal rate. The decreased buffer value of the blood with lowered CO_2 tension is shown in fig. 18. Szili (1906) slowly injected HCl into the blood of dogs until it caused death. The PH of the blood of the dog at death was about 7.05.

Owing to the prompt regulatory action of the respiratory center, the addition of acid within certain limits to the blood lowers its buffer value or bicarbonate content, and hence lowers the CO_2

tension of the alveolar air. Since more exact data on the alveolar air are available we may take them as the most reliable index of the changes in the buffer value of the blood. Barcroft (1914) measured the CO_2 tension of the alveolar air, but put most reliance on the H ion concentration of the blood at a certain CO_2 tension. In this way the buffer value is indicated as shown in Fig. 18. Barcroft estimated the H ion concentration of the blood by its effect on the dissociation of oxyhemoglobin.

Hasselbalch and Gammeltoft (1915) observed a lowering of the CO_2 tension on a meat diet. This suggests an explanation of the so-called dynamic action of protein (Lusk, 1915). On passing from a protein poor to a protein rich diet the heat output of the body is increased, even though the available energy of the food is not. Since the CO_2 tension of the alveolar air is decreased, the lung ventilation must be increased and the oxygen carried to the tissues increased as well. Perhaps the greater heat production is the direct result of this increased oxygen supply. If this be true, we may suppose that the amino acids arising from digestion of the protein increase the H ion concentration of the blood, and stimulate the respiratory center, increasing lung ventilation, this in turn increasing oxidation. Since the respiratory center is more sensitive to changes in H ion concentration than any apparatus available for measuring it, a failure to determine any increase in the H ion concentration of the blood is to be expected. It was shown by Haldane and Priestley (1905) that a 0.2 per cent rise in the CO_2 tension of the alveolar air doubles lung ventilation. A reference to Fig. 18 shows that this causes only a minute change in the PH (a change too small to measure with certainty on a carbonated fluid like the blood).

We may conclude from the foregoing that the buffer value of the blood is lowered by a meat diet. The same seems to be produced during exercise, acclimatization to high altitudes, pregnancy, diabetic coma and other conditions of acidosis. On the contrary, the experiments of Van Slyke, Cullen and Stillman (1915) indicate that the buffer value of the blood is *increased* during protein digestion. This may be due to the passage of Cl ions from the blood to the stomach with consequent increase in the bicarbonate content of the blood.

It seems probable that the PH of the blood of all animals is near that of human blood since they all contain bicarbonates. The PH of the blood of the conch (Strombus gigas) is about 7.5, that of the body fluid of the sea urchin (Cassiopea xamachana) 7.7. Although Mines supposes the PH of the blood of Pecten to be about 6.5 this was determined with indicators and does not seem to hold for the related genus, Pectenella.

Sea Water

It is maintained by A. B. Macallum (1904) that the salt composition of the blood of mammals is the same as that of the sea during the Cambrian period. In passing from the marine inveretbrates (the osmotic pressure and proportions of whose blood salts is the same as those of the sea) to the mammals, there is a gradual lowering of the osmotic pressure and of the Mg content of the blood. Macallum supposes that the evolution of the sea took the reverse direction and the osmotic pressure and Mg content was low in the Cambrian period. The osmotic pressure is constantly increased owing to the accumulation of salts carried in by rivers, which now carry a greater proportion of Ca and Mg. But the Ca is constantly being precipitated since the sea is about saturated with calcite and the Mg alone is relatively increasing. The osmotic pressure of the blood of various animals is given above. The following table gives the relative composition of the ash, Na being taken as 100:

To 100 parts Na:	Ca	K	Mg	
Sea water	3.94	3.57	11.99	(F. W. Clarke, 1911)
Medusa (Aurelia)	4.13	5.18	11.43	(Macallum, 1904)
Limulus blood	3.83	5.34	11.50	(Gotch and Laws, 1885)
Sea urchin fluid	5.45	0.24	13.27	(Mourson and Schlagdenhauffen, 1882)
Sea urchin fluid		12.00	2.20	(Griffiths, 1892)
Av'ge of Molluscs	5.43	12.20	3.07	"
Lobster blood	8.03	7.12	2.88	(Macallum)
Sipunculus (worm)	6.55	12.70	3.00	(Griffiths)
Dog's serum	2.52	6.86	.81	

The discrepancies in the proportion of K (in sea urchins, for instance) may be due partly to the fact that cells containing a larger proportion of K were sometimes included, and partly to faulty analysis.

Excised organs and cells of marine animals live for experimental periods in sea water and M. L. Lewis (1916) has shown that vertebrate tissues may proliferate in diluted sea water.

Artificial sea water may be made as follows, out of the purest reagents especially free from Ba, As and heavy metals, dissolved in water that has been redistilled with a silica condensing tube and aerated several days with outside air. The numbers denote cc of normal solutions (0.5 mol in case of bivalent salts) in a liter.

KCl	$CaCl_2$	$MgCl_2$	NaCl	$MgSO_4$	NaBr	$NaHCO_3$
10.2	22.1	50.2	48.5	57.2	0.8	2.3

After mixing, the solution is aerated with outside air several hours in order to bring the alkalinity to PH=8.1-8.2 which is the PH of the surface of the ocean. This should show a faint pink with phenolphthalein. The CO_2 tension of surface sea water is about 0.04 per cent of an atmosphere, whereas outside air averages 0.03 per cent CO_2. It is therefore possible to make the sea water slightly hyperalkaline by prolonged aeration with outside air, but the addition of animals or bacteria quickly brings it back to normal and the problem is to aerate it sufficiently. The curve of PH with varying CO_2 tension is similar to that of blood (Fig. 18) or any other bicarbonate solution.

The other constituents of sea water exist as traces. They may be present as impurities in the salts used in making artificial sea water even in excess of their concentration in natural sea water, or they may get into the water from the air or by solution of the glass vessels used to store it.

In order to investigate the significance of the composition of sea water on marine organisms the effect of changing its composition has often been studied. If the alkalinity is increased to PH=9.00 there is finally a slight precipitate of lime (calcite) and the alkalinity returns to normal, so that it is difficult to increase the alkalinity and keep it so for many days. Herbst (1898) and Loeb (1898 b) observed that an increase in the alkalinity increased the rate of development of sea urchin eggs, and O. Warburg (1810 a) found that this was accompanied by an increase in respiration of the eggs. Herbst found that Cl, Na, K, Ca and OH ions are necessary for the development of these eggs from the beginning, but SO_4, CO_3 and Mg are necessary only for later stages. Plants are less affected by changes in the composition of the sea water, but Osterhout (1912 b) has found plants that require Na. Diatoms grow better if 4 cc of n KNO_3

are added to each liter of sea water, but some algae are not benefitted by it. This high concentration of nitrates is not maintained very long owing to the presence of denitrifying bacteria, which are especially active and abundant in tropical seas.

The constituents of sea water are either nutritive or protective, or both. The H_2O, SO_4 and CO_2 are used by green plants in the manufacture of protoplasm, and they require also PO_4 and either NH_3, NO_3 or NO_2, which exist in traces in natural sea water. Traces of I, Ca, Mg, Fe, Cu, Mn, Si, etc., may serve a nutritive function to some organisms. The chief constituents of the sea water may, however, be considered protective in that they are not used up. Their presence in definite proportions is necessary in order to preserve life.

The work of Ringer, O. Loew, Loeb and others suggests that these constituents have antagonistic actions, so that a balance is necessary. Osterhout maintains that they either increase or decrease the permeability of cells. These antagonisms of the ions of sea water were demonstrated by the perfused heart of the conch (Strombus) at Tortugas, Fla. The sea water freezes at —2.03° and isotonic solutions were made as follows: 0.59 m NaCl, 0.6 m KCl, 0.36 m $CaCl_2$, and 0.38 m $MgCl_2$. Na, K and OH ions increase the rate of the heart beat and stop it in systole whereas Mg, H and Ca ions decrease the rate and stop it in diastole. These antagonisms are not perfect, however, although they are slightly more perfect in the action of these ions on the pulsations of the medusa, Cassiopea. The reason for the failure of complete antagonism is probably found in the fact that the organism is made of a number of structures, each pair of antagonistic ions having a certain antagonistic ratio for each structure, and this ratio being different for each structure. In order to obtain the same antagonistic ratio for all structures, a combination of the antagonistic pairs must be made, and the result is sea water. To use Osterhout's hypothesis, Na, K and OH ions increase the permeability and Mg, Ca and H ions decrease the permeability, but it is necessary to have all present in a certain proportion in order that the permeability of all the structures in the organism be normal. It is not sufficient to have a definite ratio between H and OH, Na and Ca, K and Mg, Na and Mg, or K and Ca.

A few protective solutions have the following percentage composition:

	$NaCl$	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$	NaH_2PO_4	Na_2HPO_4	Urea
Ringer's (frog)	.65	.014	.012	—	.02	.001	—	—
Göthlin's "	.65	.01	.0065	—	.1	.008	.009	—
Locke's (mammal)	.9	.042	.024	—	.01-.03	—	—	—
Tyrode's "	.8	.02	.02	.01	.1	.005	—	—
Führer's (sharks)	2.0	.01	.02	—	.02	—	—	2.5

The above solutions may be made nutritive by the addition of .1-.25 per cent glucose.

Many green plants will live in distilled water and need no especially protective solution except that any ion if in very high concentration must be made harmless by the presence of some antagonistic ion. Knop's solution is protective and nutritive for green plants. It is made by dissolving the following numbers of grams in 3-7 liters of water: 4 $Ca(NO_3)_2$, 1 KNO_3, 1 KH_2PO_4, 0.5 KCl, 1 $(MgSO_4 \cdot 7H_2O)$. It is probable that traces of impurities in this solution are essential since many plants contain additional elements. The analyses of Jost on the ash of a number of land plants show them to contain the following minerals, beginning with the most abundant: $CaHPO_4$, K, Mg, Mn, Na_2SO_4, Si, Cl, Fe. The P and S entered at least partly into the composition of proteins, but since anions are necessary to combine with the cations, the salts were probably phosphates, sulphates, chlorides and carbonates.

According to Stoklasa (1908) the ash of Azobacter is almost pure K_2HPO_4. Ad. Mayer (1902) used the following solution for the growth of yeast, expressed as grams dissolved in two liters: 1 KH_2PO_4, 0.1 $Ca_3\ (PO_4)_2$, 1 $MgSO_4$.

Salts of As, Cr, Zn, Cd, Ni, Co, Cu, Hg, Au and Pt are said to stimulate the growth and fermentative activity of certain fungi, when in certain minute concentrations. These salts are probably to be considered protective (affecting permeability), since the heavy metal ions are powerful antagonists to some more common cations, and only this antagonistic action explains the necessity of exactly limiting the concentration of the heavy metal.

Bacteriological media are too numerous to be considered here. The PH is very important (see papers of W. M. Clark).

APPENDIX

CHEMICAL SUMMARY

The elements contained in every living cell are as follows:

Element	Symbol	Atomic weight	Valence
Hydrogen	H	1.008	1
Carbon	C	12	4
Nitrogen	N	14.01	3, or 5, or 4
Oxygen	O	16	2 4 (6 or 12?)
Phosphorus	P	31.04	3 5
Sulphur	S	32.07	2, 4, 6
Chlorine	Cl	35.46	1 (2, 3, 4, 5?)
Sodium	Na	23	1
Potassium	K	39.1	1
Magnesium	Mg	24.32	2
Calcium	Ca	40.07	2
Iron	Fe	55.84	3 2

Other elements sometimes present are as follows:

Element	Symbol	Atomic weight	Valence	
Fluorine	F	19	1	(2, 3, 4,5?)
Bromine	Br	79.92	1	
Iodine	I	126.92	1	
Aluminium	Al	27.1	3	
Silicon	Si	28.3	4	
Manganese	Mn	54.93	3, 2, 7	
Copper	Cu	63.57	2 or 1	

None of these elements exist as simple atoms in the cell. The chief compound is water, HOH. It seems probable, from the high surface tension, specific heat and dielectric constant of water, that some molecules have combined (polymerized) to form $(HOH)_n$. It is even believed that some of these aggregates are colloidal particles, and cause the blue color of the sea, but it seems more probable that this blue light is dispersed by water molecules, as the blue sky is produced by dust particles on drops of water in the air. Gases dissolve in water in the form of O_2, N_2 or H_2. Many of the elements exist in solution as salts, formed by the combination of an acid and a base. Na, K, Mg, Ca, Al, Mn and Fe combine with O, forming oxides, which combine with water forming hydroxides, which are bases. F, Cl, Br and I combine with H to form acids and Si, P, S, C, N and O may enter into the composition of acids.

The compounds of carbon are so complex and numerous as to form a special group, organic compounds. This is subdivided into the open chain or aliphatic series and the ring compounds or aromatic series.

The paraffins are compounds of carbon and hydrogen in open chain. Starting with methane, CH_4, a combination of two molecules gives ethane, C_2H_6, because two hydrogen atoms are eliminated and the valences thus freed are used to connect the two carbon atoms. In a similar way propane, C_3H_8, and butane, C_4H_{10}, and an indefinite number of higher compounds are formed.

In the paraffins all of the valences of the carbon atoms are filled and no more hydrogen atoms can be attached to the molecule without splitting it between two carbon atoms, and therefore these compounds are said to be saturated. In the unsaturated hydrocarbons, however, more hydrogen atoms may be attached without splitting the molecule, which is thereby converted into a saturated compound. There are no free valences in the unsaturated compounds but two carbon atoms are united by more than one bond, and by opening up one bond two free valences are formed for the attachment of two hydrogen atoms. Thus in ethylene, C_2H_4, there is a double bond between the carbon atoms and acetylene, C_2H_2, there is a triple bond between the two carbon atoms.

The first products of the oxidation of the paraffines are the alcohols, methyl alcohol, CH_3OH, ethyl alcohol, C_2H_5OH, propyl alcohol, C_3H_7OH, and so on. Alcohols may combine with bases, but where water is formed by the combination the compound may decompose again. If the presence of water be eliminated the compound is relatively stable. Thus when metallic sodium is dropped into ethyl alcohol, sodium ethylate is formed with the evolution of hydrogen.

$$C_2H_5OH + Na = C_2H_5ONa + H \text{ and } 2H = H_2.$$

The combination of an alcohol with an acid forms an ester, thus:

$$C_2H_5OH + H_2SO_4 = C_2H_5HSO_4 + H_2O.$$

If an ester is warmed with a strong base, the base combines with the acid and the alcohol is set free, a process known as saponification, thus:

$$C_2H_5HSO_4 + Ca(OH)_2 = C_2H_5OH + CaSO_4 + H_2O.$$

The alcohols with one OH group, like the ones given above, are called monatomic. The highest monatomic alcohol found in cells is cholesterol, $C_{27}H_{45}OH$, a waxy substance, insoluble in water and crystallizing in plates.

Alcohols with three OH groups are called triatomic. The one found in cells is glycerol, $C_3H_5(OH)_3$, which is soluble in water, and its esters with fatty acids are called fats.

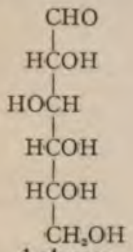
By oxidation of alcohols we obtain aldehydes. Methyl alcohol gives form-aldehyde, thus: $2CH_3OH + O_2 = 2HCHO + H_2O$. In the aldehyde group, CHO, the oxygen is united with the carbon by a double bond, which makes the aldehyde capable of further oxidation, in fact it has such an affinity for oxygen as to be a mild reducing agent. The product of such an oxidation of an aldehyde is a fatty acid and the aldehyde is named for the acid that it forms and not for the alcohol from which it is formed, where there is a different name for the alcohol and acid. Thus we have methyl alcohol, formaldehyde and formic acid and we have ethyl alcohol, acetaldehyde and acetic acid, but propyl alcohol, propylaldehyde and propionic acid.

The CHO group of the aldehyde contains the terminal carbon atom of the chain. If an intermediate carbon atom of the chain bears the double bond we have a CO group, and the substance is called a ketone. Thus, CH_3CH_2CHO is propyl aldehyde, but CH_3COCH_3 is dimethyl ketone (acetone).

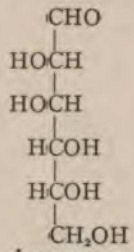
The carbohydrates are aldehydes or ketones of polyatomic alcohols, in which the hydrogen and oxygen are usually in the proportion to form water. Aldehydes have a tendency to polymerize or combine to form larger molecules. Formaldehyde in alkaline solution polymerizes to form a series of sugars with six carbon atoms. This seems also to be the mode of formation of sugar in the green plant. The carbonic acid, H_2CO_3, is reduced by the action of sunlight and the fluorescent chlorophyll to HCHO, the oxygen removed from the carbonic acid combines with water to form hydrogen peroxide, which is immediately decomposed by the catalase of the cell with the liberation of O_2. The formaldehyde would destroy the cell if it were not immediately polymerized by the action of the green bodies, chloroplasts.

The most important sugars are the hexoses or those with six carbon atoms, which are asymmetrical. The carbon atom has four valences and if a different kind of atom or group is attached to each of these valences, the molecule is asymmetrical, that is to say, no point, line or plane of symmetry exists. It is then possible to arrange the groups around the carbon atom in two different ways so that one form of molecule will be the mirrored image of the other. Such asymmetry can be detected because a molecule containing one asymmetric carbon atom rotates the plane of polarized light to the right or the left. If a molecule contains two asymmetric carbon atoms, both atoms may rotate in the same direction or in opposite directions, and if the rotation is equal in opposite directions the molecule appears inactive to polarized light. There are often obtained mixtures of equal numbers of dextro-rotatory and laevo-rotatory molecules, the mixture does not affect polarized light and is called racemic.

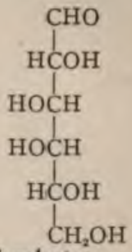
The hexoses that are readily assimilable by mammalian cells are:



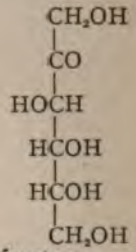
d-glucose
(dextrose



d-mannose



d-galactose



d-fructose
(laevulose)

This is the nomenclature of Fischer, based on structural formulae. All of these sugars are aldehydes (aldoses) and rotate polarized light to the right, except d-fructose (a ketose) which is usually called laevulose because it rotates polarized light to the left.

On the oxidation of 1 g. glucose about 3.74 calories of heat are produced.

Glucose, fructose and galactose, when reduced, are converted into the alcohols, sorbite, mannite and dulcite. Glucose on mild oxidation, yields gluconic acid, with the COOH group formed from the CHO group. On further oxidation another COOH group is formed on the other end of the chain, and thus the dibasic acid, saccharic acid is formed. Glucose, as well as other hexoses, is easily transformed into lactic acid without oxidation. One molecule of glucose yields two molecules of lactic acid, $CH_3CHOHCOOH$, when acted on by lime in sunlight or by bacteria, or other living cells.

As aldehydes or ketones, the hexoses are reducing substances, but this property may be lessened spontaneously, in solution, by what is called lactone formation. In this, the double bond between one carbon and oxygen atom, to which the reducing character is due, opens out and one bond becomes applied to a distant carbon atom in exchange for an OH group.

Glucose may combine with other substances, forming glucosides. This combination is usually preceded by lactone formation. Two hexose molecules may combine by the opening out of a double bond and the elimination of one molecule of water, forming a disaccharide molecule. Thus glucose and glucose form maltose, glucose and galactose form lactose, and glucose and fructose form saccharose or cane sugar. One gram of a disaccharide on oxidation produces 3.95 calories of heat.

Pentoses, with five carbon atoms, are found in many plant cells.

Polysaccharides are formed by the combination of simple sugar molecules. Gums, pectens and mucilages are formed of monosaccharides, and starch, dextrin, glycogen and cellulose are formed of glucose. The molecular weight of starch is estimated at between 1260 and 32400. Its osmotic pressure is so small that it appears to be zero.

By the oxidation of the CHO group of the aldehydes the COOH group of the organic acids is obtained. The open chain acids are called fatty acids and bear the same name as the aldehydes from which they are formed. In the series, formic, acetic, propionic, butyric, valerianic and caproic, each member has one more carbon atom than the one preceding. The fatty acids most common in animal cells have even numbers of C atoms. These acids are butyric, caproic, caprylic, capric, lauric, myristic, palmitic and stearic. The solubilities of these acids in water decrease as the number of carbon atoms increases, the higher members of the series being insoluble.

In the same way that unsaturated hydrocarbons differ from paraffines in the presence of double or treble bonds between adjacent carbon atoms, so the unsaturated fatty acids differ from those given above. The unsaturated fatty acids, oleic, linoleic have respectively 1 and 2 double bonds. Linolenic has 3 double bonds. These unsaturated acids may take up oxygen and become saturated, and in this way they act as reducing substances.

The oxy-acids contain more oxygen than the normal acids. Propionic acid is CH_3CH_2COOH whereas oxypropionic, usually called lactic acid, is $CH_3CHOHCOOH$.

Keto acids contain the ketone group, and also the COOH group.

Fatty acids combine with bases to form salts, which in case of the higher members of the series are called soaps. The soaps formed by action of alkaline earths on the higher fatty acids are insoluble in water whereas the lower fatty acids form soluble soaps with these bases. Alkali soaps hydrolize in water, forming a fine emulsion of fatty acid that makes the solution opalescent.

Fatty acids combine with ammonia, but the compound may be of either of two forms. With ammonium hydrate, which is a true base, they form ammoniacal soaps, but with dry ammonia and a dehydrating agent they form acid amides containing one less molecule of water. In ammoniacal soaps N is pentivalent in the group $COONH_4$, whereas in amides nitrogen is trivalent in the group $CONH_2$.

The esters of fatty acids with alcohols are called fats. The triatomic alcohol glycerol is the most common but the monatomic alcohol cholesterol and other alcohols are also found.

Since glycerol is a triatomic alcohol, it can combine with one, two or three fatty acid molecules, forming mono-, di-, and triglycerides, of which the last is the most common. Fat globules found in cells are usually mixtures of triglycerides and fatty acids. In the analysis of fats, the acid number denotes the content of free fatty acids, the saponification number, or combining power with hot KOH, is an index of the average of molecular weight of each fatty acid divided by its valence, and the iodine number is an index of the content in unsaturated fatty acids. The percentage of volatile fatty acids is usually determined, but the separation of all of the fatty acids involves much labor.

The oxidation of 1 g. fat may yield as much as 9.5 calories of heat.

Mono or diglycerides may combine with other substances by means of the remaining alcohol groups. Such compounds are included under the loose term, lipoids, but have been called by Leathes phospholipines, galactolipines and lipines. Phospholipines are diglycerides in which the third alcohol group is combined with phosphoric acid. Since phosphoric acid is trivalent it can combine with two other substances. In lecithin it combines with only one other substance, the organic base, choline. The phospholipines are unstable and are partly decomposed and oxidized by extraction with hot solvents in the presence of air, and hence their composition has not been entirely settled in all cases.

In galactolipines, galactose takes the place of glycerol. Lipines contain nitrogen but no carbohydrate or phosphoric acid. From fatty acids and ammonia may be prepared not only acid amides, but also amino acids (lactic acid with NH_3 and a dehydrating agent forms alanine). In the mono-amino acids obtained from mammalian cells the NH_2 group is attached to the carbon atom next to the COOH group. These acids are called alpha amino acids. In the diamino acids, an additional NH_2 group is attached to some other carbon atom of the chain.

The amino acids have both acid and basic properties and are therefore called amphoteric. In the diamino acids the basic property predominates. Amino acids may be derived from dibasic acids, containing two COOH groups and in this case the acid property markedly predominates.

The proteins are compounds of amino acids in which the NH_2 group of one molecule has reacted with the COOH group of another. The complete structure of none of the proteins is known, but many amino acids have been separated from the decomposition products, among which are: The monoamino acids, amino acetic or glycine, aminopropionic or alanine, amino isovalerianic or valine, and aminoisocaproic or leucine. The dibasic amino acids are aspartic and glutamic acids. The diamino acids are lysine, arginine, and diaminotrioxydodecoic acid. Cystine contains two atoms of sulphur. Other amino acids contain cyclic compounds, which will now be considered.

Whereas in the compounds thus far considered the carbon atoms are arranged in an open chain, in the cyclic compounds they are in the form of a closed ring. The most important of these rings is the benzol ring containing 6 carbon atoms. Although this ring has only 6 H atoms there seem to be no double bonds between the carbon atoms, or if there are double bonds they do not behave as the double bonds of the unsaturated open chain compounds. In benzol, C_6H_6, each C atom is united to one hydrogen atom. The substitution of one or more hydrogen atoms by other atoms or groups gives rise to a host of compounds. When only one hydrogen is substituted it is immaterial which carbon atom it is attached to, but by its presence the other carbon atoms lying at different distances from the first one are designated as ortho, meta and para, and groups that may subsequently be attached to them are in the ortho, meta, or para position.

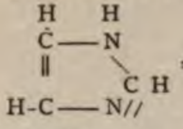
Of the mono-substitution products of benzol we have aniline, $C_6H_5NH_2$, benzol-sulphonic acid, $C_6H_5SO_3H$, phenol, C_6H_5OH, toluol, $C_6H_5CH_3$, benzyl alcohol, $C_6H_5CH_2OH$, benzaldehyde, C_6H_5CHO, and benzoic acid, C_6H_5COOH.

Of the di-substitution products we have catechol (ortho-hydroxyphenol), resorcinol (meta-hydroxyphenol), hydroquinone (para-hydroxyphenol), salicylic acid (ortho-hydroxy benzoic acid), and tyrosin (para-hydroxyphenylalanine). Tyrosin is an amino acid found in proteins.

Some tri-substitution products are pyrogallol, homogentisic acid, picric acid and adrenaline. Adrenaline is secreted by cells of the adrenal medulla and is a specific stimulant (or in some cases inhibitor) of smooth muscle cells, although its action is supposed not to be on the whole cell surface but on a theoretical receptor substance.

The pyrrol ring contains 1 nitrogen and 4 carbon atoms and is contained in proline and oxyproline, amino acids found in proteins. Tryptophane, another amino acid found in proteins, contains a combination of benzol and pyrrol rings and may be transformed into indol and skatol by the action of bacteria.

The imidazol ring contains three carbon and two nitrogen atoms



= Imidazol ring.

and is contained in histidine, an amino acid found in proteins.

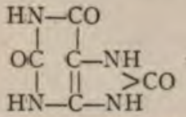
When protein is split into amino acids by any method, not all of the protein is thus accounted for.

Now that the building stones of the proteins have been discussed, it is well to consider a few characteristics of these interesting substances.

Proteins consist of the same amino acids but in different proportion. Glycine is absent from some proteins. Tyrosine and tryptophane are absent from gelatine.

Proteins consisting almost entirely of amino acids are called simple proteins to distinguish them from those combined with other substances. In oxyhemoglobin, a simple protein is combined with hematin, an iron containing organic compound loosely combined with one molecule of O_2.

The nucleins that have been isolated from the nuclei of some cells are composed of a simple protein and nucleic acid. The nuclein may combine with more protein to form nucleo-protein. Nucleic acid contains phosphoric acid and one or more purine bases, adenine or guanine, allied to uric acid.



= uric acid.

The glycoproteins, such as the mucins, contain a high percent of carbohydrate groups, which are also found in nucleic acid.

The phosphoproteins, vitellin, casein, contain phosphoric acid that is not in the form of nucleic acid or lecithin.

The lecitho-proteins are hypothetical, although both lecithin and protein exist in the crystalline yolk bodies of the frog's egg. Hoppe Seyler considered such substances to be true chemical compounds but others have regarded them as mixtures. One argument against their being mixtures is that the yolk bodies are clear and not turbid as colloidal gels or sols containing lecithin are.

The molecular weight of simple proteins has been estimated as varying from 14,000 to 30,000, whereas that of oxyhemoglobin is about 16,000.

Proteins have both acid and basic characters since they contain both COOH and NH_2 groups. Their acid character predominates when in alkaline solution and basic character predominates when in acid solution. Proteins are precipitated by heavy metals and the color bases of many dyes and by the same acids that precipitate alkaloids, namely tannic, metaphosphoric, picric, phosphomolybdic, phosphotungstic, tri-iodo-hydriodic, chromic, bichromic, and the color acids of many dyes.

Certain bacteria have the power of splitting off ammonia from the amino acids of proteins, a process known as deamination. If the deamination is accompanied by hydrolysis oxyacids are formed, whereas if reduction takes place fatty acids are formed. In the eggs of certain fish fats are formed from proteins. The glycerine necessary in the process arises from proteins and may possibly arise in part in the following manner. In the conversion of glucose into lactic acid glyceric aldehyde is formed, which by reduction becomes glycerol.

ABBREVIATIONS USED IN LITERATURE LIST

AA	Anatomischer Anzeiger.
AAP	Arch. f. Anat. Physiol. (Physiol Abt.) (Bu Bois Reymond, Engelmann).
AB	Archiv de Biologie (Van Beneden).
ABt	Archiv für Botanik.
AnB	Annals of Botany.
AC	Ann. Chem. u. Pharm. (Liebig) (= An. Pm.)
ACJ	American Chemical Journal.
ACP	Ann. d. Chim. et Phys. (= A. Phys.).
AE	Arch. Entwicklungsmech. (Roux).
AEP	Arch. f. Exp. Path. u. Pharmakologie.
AF	Archivio di Fisiologia.
AGP	Arch. gesammt. Physiol. (Pflüger).
AH	Archiv für Hygiene.
AIB	Archives Ital. d. Biol.
AIM	Archives of Internal Medicine.
AIP	Arch. Internat. de Physiol.
AIPd	Arch. Internat. d. Pharmacodynam.
AIPr	Ann. d. l'Ins. d. Pasteur.
AJA	American Journal of Anatomy.
AJB	American Journal of Botany.
AJDC	Am. Jl. of Diseases of Children.
AJMS	Am. Jl. of the Medical Sciences.
AJP	Am. Jl. of Physiology.
AJS	American Journal of Science.
AK	Archiv für Kinderheilkunde.
AKG	Arb. a. d. kais. Gesundheitsamt.
AMBG	Annals Missouri Bot. Garden.
AN	American Naturalist.
ANd	Arch. Neerland.
ANYA	Ann. N. Y. Acad. of Science.
APk	Ann. d. Physik (Poggendorf, Wied., Drud.) (= Jl. Pk., N. J. Pk., A. Pk. C.)
APm	Archiv für Pharm.
AR	Anatomical Record.
ASB	Arch. d. Sci. Biol.
ASNZ	Ann. d. Sci. Natur. et Zool.
ASPN	Arch. Soc. Phys. Nat. Geneva.
AUG	Ann. d. l'Univer. de Grenoble.
AZ	Archiv für Zellforschung.
AZEG	Arch. d. Zoolog. Experim. et Gener.
BARB	Bull. d. l'Acad. Roy. Belgique.
BASC	Bull. Acad. Sc. Cracow. Math. Nat.
BB	Biological Bulletin.
BBF	Bull. U. S. Bureau of Fisheries.
BBS	Bull. U. S. Bureau of Standards.
BC	Biologisch. Centralblatt.
BCB	Biochemical Bulletin.
BCPP	Beiträge Chem. Physiol. u. Pathol. (Hofmeister).

BDBG	Berichte d. deutsch. bot. Gesell.
BDCG	Ber. d. deutsch. chem. Gesellschaft.
BG	Botanical Gazette.
BGS	Bull. U. S. Geological Survey.
BHL	Bull. Hyg. Lab. U. S. Pub. Health & M. H. S.
BIOM	Bull. Inst. Oceanograph. Monaco.
BJ	Biochemical Journal.
BKW	Berliner klin. Wochenschrift.
BMJ	British Medical Journal.
BMCZ	Bull. Museum Comp. Zool. Harvard.
BSC	Bull. Soc. Chim.
BTBC	Bull. Torrey Botanical Club.
BWA	Berichte der Wiener Akad.
BVGW	Ber. Verh. Gesell. Wissens. Leipzig.
BZ	Biochemische Zeitschrift.
BZg	Botanisches Zeitung.
CIWP	Carnegie Inst. Wash. Publication.
CRA	Compt. Rend. Acad. Sc. Paris.
CRB	Compt. Rend. Soc. Biol. Paris.
CZ	Chemische Zeit.
DAKM	Deutsch. Archiv klin. Medicin.
DMW	Deutsch. med. Wochenschrift.
EAPP	Ergebnisse allg. Path. u. path. Anat.
EP	Ergeb. d. Physiologie (Asher & Spiro).
F	Flora.
GCI	Gazetta Chim. Ital.
IRGH	Internat. Rev. ges. Hydrobiologie.
IZPB	Int. Zeitsch. physikochem. Biologie.
JAB	Journal of Animal Behavior.
JACS	Jl. of the Amer. Chemical Society.
JAMA	Jl. Amer. Medical Association.
JAP	Jl. of Anatomy and Physiology.
JBC	Journal of Biological Chemistry.
JCET	Jl. Coll. Engineering I. U. Jap. Tokyo.
JCNP	Jl. Comparative Neurol. & Psychol.
JCP	Jl. Chim. et Phys.
JCS	Jl. of the Chemical Society.
JCSJ	Jl. Coll. Sc. Imp. Univ. Jap. Tokyo.
JEM	Journal of Experimental Medicine.
JEZ	Journal of Experimental Zoology.
JHHB	Johns Hopkins Hosp. Bulletin.
JID	Journal of Infectious Diseases.
JLCM	Jl. of Laboratory & Clin. Medicine.
JLS	Journal of the Linnean Society.
JMBA	Jl. Marine Biol. Assn. Unit. King. (n. s.)
JMR	Journal of Medical Research.
JP	Journal of Physiology.
JPm	Jl. de Pharmacie.
JPq	Jl. de Physique, Theor. et Appl.
JPB	Jl. Pathol. Bakteriol.
JPC	Journal of Physical Chemistry.
JPkC	Jl. für prakt. Chemie.
JPET	Jl. Pharmacol. Exp. Therapeutics.
JPP	Jl. de Physiol. et de Pathol. General.
JWAS	Jl. Washington Acad. Sc.
JWB	Jahrbucher wissenschaftl. Bot.
KCB	Kolloidchemische Beihefte.
KZ	Kolloid Zeitschrift.

LJ	Landwirtschaftliche Jahrbücher.
MARB	Memoirs Acad. Roy. Belgique.
MC	Monatshefte Chem.
MKW	Münchener klinische Wochenschrift.
MMW	Münchener medizinische Wochenschrift.
MRR	Medical Review of Reviews.
NR	Natur. Rundschau.
NVS	Nordske. Vedenskabs. Schrift.
PANS	Proc. Acad. Nat'l. Science, Phila.
PBS	Proc. Boston Soc. Natural History.
PCPS	Proc. Cambridge Philosophical Soc.
PM	Philosophical Magazine.
PrM	Presse Medical.
PNAS	Proc. National Acad. Science.
PNL	Pub. Nutrition Lab. Carnegie Ins. W.
PR	Physical Review.
PRS	Proc. Royal Society of London.
PSEB	Proc. Soc. Exper. Biol. & Medicine.
PSM	Popular Science Monthly.
PT	Philos. Transactions Roy. Soc. London.
PW	Plant World.
PZ	Physikalische Zeitschrift.
QJEP	Quart. Jl. Experimental Physiology.
QJM	Quarterly Journal of Medicine.
RTCP	Rec. Traveaux Chim. Pays Bas.
RRAL	Rendiconti R. Acad. dei Lincei.
S	Science (n. s.).
SAP	Skandinavisches Archiv Physiologie.
SKPA	Sitzungsb. kais. Pruss. Akad. Wiss.
SPRD	Sci. Proc. Roy. Dublin Society (n. s.).
SWA	Sitzungsber. Wiener Akad.
TAAP	Trans. Amer. Acad. Physicians.
TAES	Trans. Amer. Electrochem. Soc.
TCA	Trans. Connecticut Academy.
TCS	Trans. Chemical Society.
TLA	Traveaux d. l'Lab. d. Archachon.
TFS	Transactions of the Faraday Soc.
UPMB	Univ. Penna. Medical Bulletin.
UCPP	Univ. Cal. Publications (Physiol.).
UMS	Univ. Minn. Studies (Phys. Sc. & Math.).
VGDN	Verh. Ges. deutsch. Nat'f. u. Arzte.
VNGZ	Vier'j's. Nat. Gesellsch. Zurich.
VNVB	Verhandl. Nat. Verein, Brünn.
VPMG	Verh. phys.-med. Gesellsch. Wurzburg.
VKAW	Ver. Kon. Acad. Wetens., Amsterdam.
WKW	Wiener klinische Wochenschrift.
ZAC	Zeitschrift anorganische Chemie.
ZAP	Zeit. allgemeine Physiologie.
ZB	Zeitschrift für Biologie.
ZBk	Zentralblatt für Bakteriologie.
ZBt	Z. Botanik.
ZEC	Zeitschrift für Elektrochemie.
ZEP	Zeit. f. exper. Pathol. u. Therapie.
ZET	Zeit. f. exper. Ther.
ZH	Zeitschrift für Hygiene.
ZI	Zeitschrift für Immunität.
ZIM	Zent. f. innere Med. (= Cent. klin. M. = C. I. M.)
ZJ	Zoolog. Jahrbucher (Zool. Physiol. Abt.).

ZK	Zeitschrift für Kinderheilkunde.
ZKlM	Zeitschrift für klinisch. Medizin.
ZN	Zeit. für Nervenheilkunde (Deutsche).
ZP	Zentralblatt für Physiologie.
ZPa	Zeitschrift für Pathologie.
ZPk	Zentralblat für Physik.
ZPC	Zeitschr. für physiolog. Chemie.
ZPkC	Zeits. für Physikalische Chemie.
ZRM	Zeitschrift für Rationell. Medizin.
ZWM	Zeit. für wissenschaft. Mikroskopie.
ZWZ	Zeit. für wissenschaftlich. Zool.

LITERATURE LIST

Abegg, Auerbach & Luther 1911 "Messungen Elektromot. Krafte gal. Kette." Halle.
Abderhalden 1898 Analysis of mammal blood. ZPC xxv 65.
1909 Milk-droplet membranes. ZPC lix 13.
1910 "Handbuch d. Biochem Arbeitsmethoden." Berlin.
1911 "Biochem. Handlexikon." Berlin.
—— & Gigon 1907 Yeast (splitting dipeptids) inhibited by l-amino acids. ZPC lii 251.
—— & Guggenheim 1908 Shaking enzymes. Asymmetry. ZPC liv 331.
—— & Hall 1908 "Text Book of Physiol. Chem." N. Y.
—— & Pringsheim 1909 Asymmetry and enzyme action. ZPC lix 249.
Abel, Rowntree & Turner 1914 Diffusible constituents of blood. JPET v 275.
Abl 1907 Anesthetic and electrode potential. Dissertation, Bonn.
Acree & Johnson 1907 Enzymes displace equilib. pt. ACJ xxxviii 258.
Adrian 1914 All-or-none law. JP xlvii 460.
Agazotti 1906 PH of blood at low atm. pressure. RRAL (5) xv 481.
Albutt 1910 Blood viscosity. QJM iv 342.
Alcock & Lynch 1911 K in nerve. JP xliii 107.
Alexander & Cserna 1913 Narcosis and brain respiration. BZ liii 100.
Allee 1914 Rheotaxis and KCN. JEZ xvi 397.
—— & Tashiro 1914 Rheotaxis and CO_2 production. JAB iv 202.
Allemand 1912 PH and rennin action. BZ xlv 346.
Allen, F. 1916 Diabetes. JAMA lxvi 1525.
Allen, G. 1915a Bile and surface tension of urine. JBC xxii 503.
1915b Reversibility of rheotaxis. BB xxix 111.
Allen, E. & Nelson 1910 Diatom culture. JMBA viii 421.
Allen, H. 1913 "Photoelectricity: the Lib. of Electrons by Light." London.
Armstrong, E. 1903 Enzymatic splitting and synthesis of sugars. JCS lxxxiii 1305.
1904 Action of invertase inhibited by fructose. PRS (B) lxxiii 500, 516, 526.
Armstrong, H. 1908 Properties of water. PRS (A) lxxxi 80.
Arrhenius 1887 Ionization. ZPkC i 631.
1889 Osmotic pressure and vapor pressure. ZPkC iii 115.
1892 Usually ions decrease and non-electrolytes increase viscosity of water. ZPkC x 51.
1901 "Lehrbuch der Elektrochemie."
Asher & Karaulow 1910 Permeability of salivary cells to sugar. BZ xxv 36.
—— & Spiro 1902-1915 Ergebnisse der Physiologie. Wiesbaden.
—— & Waldstein 1906 Increased blood pressure not diuretic. BZ ii 1.
Atkins 1910a Δ Marine vertebrates. BJ v 213.
1910b Beans not semipermeable. SPRDS xii 35.
1910c Δ Plant juices. SPRDS xii 463.
Aubert 1912 Thermo-osmose. ACP xxvi 145, 551.
Auer & Meltzer 1916 $MgSO_4$ anesthesia. JPET xxiii 641.
Auerbach 1912 EH of N/10 Cal. Elec. = .337, 0°-25°. ZEC xviii 13.
1912 PH of N/10 $NaHCO_3$ = 8.3 at 25°. AKG xxxviii 242.
1913 PH for Trypsin on Peptone = 8.3 = $NaHCO_3$. BZ xlviii 425.

Austrian 1911 Case of visc. blood = 13.6. JHHB xxii 9.
Bach & Chodat 1904 Oxygenase and peroxidase. BDCG xxxvi 606.
Bachman 1912 Δ of amphibian eggs. AGP cxlviii 141.
—— & Runnstrom 1909 Δ of frog's eggs = .045. BZ ccii 290.
  1912 Unfertilized frog's egg swells more than fertilized. AGP cxliv 287.
—— & Sundberg 1912 Δ of tadpoles. AGP cxlvi 212, 287.
Bachmann 1912 Ultramicroscopy of soaps. KZ xi 145.
Bachmetjew 1900 Undercooling of insects. ZWZ lxvii 529.
Bäyer 1902 O_2-Need of nerve. ZAP ii 169.
Bainbridge 1902 Bile salts increase secretion and lymph production of liver. JP xxviii 204.
——, Collins & Menzies 1913 Frogs' glomeruli secrete isotonic urine. PRS (B) lxxxvi 353.
Baltzer 1911 Mitosis. AE xxxii 500.
Bancels, des 1909 Effect of ions on Polarization. CRA cxlix 316.
Bancroft 1906 Reversal of galvanotropism. UCPP iii 21, JP xxiv 444.
Banta 1912 Development and respiration. PSEB ix 104.
Barcroft 1914 "The Respiratory Function of the Blood." Cambridge University Press.
—— & Brodie 1905 Osmotic work and kidney metabolism. JP xxxiii 52.
—— & Camis 1909 Dissociation of oxyhemoglobin. JP xxxix 118.
—— & Hill 1910 Effect of temperature on dissociation of oxyhemoglobin. JP xxxix 81.
—— & Straub 1910 Kidney metabolism and osmotic work. JP xli 145.
Barker, Hirschfelder & Bond 1910 Electrocardiogram. JAMA lv 1350.
Banta & Gortner 1915 Displacement of organ forming substance. JEZ xviii 433.
Barger 1904 Microtensimeter. TCS lxxxv 286.
—— & Starling 1915 Adsorption of iodine. TCS cvii 411.
Barlow 1896 Infusion of sugar causes greater hydremia than urea. JP xix 418.
Bartell 1911 Permeability. JPC xv 659.
  1912 Diameter of pores and osmose. JPC xvi 318.
  1914 Negative osmose. JACS xxxvi 646.
—— & Hocker 1916 Negative osmose and diffusion emf. JACS xxxviii 1029, 1036.
Bartelzko 1909 Freezing and plasmolysis. JWB xlvii 57.
Barns 1889 Suspensoids precipitated by ions not by electrolytes. AJS xxxvii 122.
Bataillon 1910 Parthenogenesis with foreign sperm. AZEG (5) vi 101.
  1911 Parthenogenesis by pricking and electricity. CRB lxx 562.
  1912 Parthenogenesis. ASNZ (9) xvi 249.
Batelli & Stern 1907 Cell respiration. JP ix 1, 34, 228, 410, 737.
  1909 Oxidation. BZ xxi 487, xxxiv 263.
  1910 Oxidation. BZ xxiii 145.
  1913 Anesthetics precipitate protein. BZ lii 226, 253.
Bates 1912 Faraday = 96500 coulombs. Thesis, U. of Ill.
Bauer 1913 Model of electric organ. ZEC xix 590.
Bayliss 1906 Adsorption and electric charge. BJ i 173.
  1908 Frog's skin, or bag of Congo red solution acts as electric rectifyer. BZ xi 226.
  1909 Osmotic pressure of Congo red. PRS (B) lxxxi 269.
  1910 Membrane hydrolysis. KZ vi 23.
  1911 Permeability and polarization. PRS (B) lxxxiv 245.
  1913 Asymmetry and enzyne action. JP xlvi 236.
  1915a "Principles of General Physiology." Longmans.
  1915b Enzymes may act in non-solvent media. JP l 85, S xlii 509.
  1916 Viscosity and blood pressure. JP l (p. xxiii).

Beard & Cramer 1915 Surface tension and ferments. PRS (B) lxxviii 575.
Beccari 1915 K within physiol. limit stimulating to muscle. AIB lxiii 293.
Bechhold 1904a "Irregular Series" of ionic and colloidal precipitation, Bacteria are anodic. ZPkC xlviii 385.
1904b Electrolytic solution tension and precipitating power. ZPkC xlviii 406.
1907 Permeability of membranes. ZPkC lx 257.
1908 Ultrafilter. ZPkC lxiv 328.
1912 "Die Kolloide in Biologie und Medizin." Dresden.
—— & Ziegler 1906 Diffusion in gels. ZPkC lvi 105.
1910 Solubility of uric acid in serum. BZ xxiv 146.
Beddard 1902 Dyes not excreted by glomeruli of frog's kidney, but by tubules. JP xxviii 20.
Begun, Hermann & Münzer 1915 Alv. CO_2 (Plesch) 6%. Drank 20 g HCl and Alv. CO_2 fell 1%. BZ lxxi 255.
Bein 1899 Effect of membrane on transport numbers. ZPkC xxviii 439.
Benecke 1898 Ca-free water causes cells to fall apart. JWB xxxii 474.
Benedict & Cathcart 1913 Muscular work. PNL (No. 187).
Benedikt 1906 PH of diabetic blood. AGP cxv 106.
Beniasch 1912 Acid agglutination. ZI xii 268.
Bennett & Cole 1912 Regeneration of storage cells. TAES.
Benson 1902 Adsorption on foam. JPC vii 532.
Benson & Wells 1910 Antolysis. JBC viii 61.
Benrath & Sachs 1905 Formation of HCl by stomach. AGP cix 460.
Bequerel 1901 Seeds survive complete dessication. CRA cxxxix 1721.
Berczeller 1913 Change of surface tension of colloids. BZ liii 232.
1914 Surface tension of alkaline solutions of lecithin increased by ether or chloroform. BZ lxvi 226.
—— & Csaki 1913 Surface tension of alkaloids. BZ liii 238.
Berg 1912 Muscle contraction. BCB ii 101.
Bergeim 1914 Origin of gastric HCl. PSEB xii 21.
Berkeley & Hartley 1904 Osmotic pressure of sugar. PRS lxxiii 436.
1906 Osmotic pressure of conc. sol. PT (A) ccvi 481.
Bernoulli 1913 Sulphates and nitrates temporarily reduce effect of bromide intoxication. AEP lxxiii 353.
Bernstein, E. & Simons 1911 Meiostagmine reaction. AJMS cxlii 852.
Bernstein, J. 1866 Polarizability of nerve lessened by stimulants. AP 596.
1900 Imitation of ameba. AGP lxxx 628.
1901 Surface tension theory of muscle contraction. AGP lxxxv 271.
1902 Theory of bioelectric currents. AGP xcii 521.
1908 Temperature coefficient of muscle contraction and surface tension is negative and of swelling positive. AGP cxxii 129, cxxiv 462.
1910 Thermo. currents of muscle. AGP cxxxi 589.
1912 "Elektrobiologie." Braunschweig. Vieweg.
1914 Muscle contraction. AGP clvi 299.
1915 Swelling causes no contraction unless previously stretched or twisted. AGP clxii 1.
—— & Von Tschermak 1906 Electric organ. AGP cxii 439.
Bert, Paul 1878 La pression barometique. Paris, Masson.
Berthelot & Gaudechon 1910 Photosynthesis of carbohydrates. CRA cl 1690.
—— & Jungfleisch 1872 Partition coefficient independent of concentration. ACP (4) 396, 408.
Bethe 1908 Van't Hoff solution. Toxicity of sugar. AGP cxxiv 541.
1909 Movements of medusa and perm. to $H^{\cdot}$ and OH'. AGP cxxvii 219.
1911 Electroendosmose. MMW lviii 168.
1914 Blood corpuscles of ascidians more permeable to acid than to basic dyes. BIOM (No. 284).
1916 Stimulation due to $H^{\cdot}$. AGP clxiii 147.

—— & Toropoff 1914 Change of reaction by electroendosmose. ZPkC lxxxviii 686.
Beutner 1912a Colloidal and osmotic swelling. BZ ix 280.
  1912b Concentration cells. BZ xlvii 73.
  1913a Concentration cells. ZEC xix 319.
  1913b Concentration cells. JPC xvii 344.
  1913c Colloidal and osmotic swelling. BZ xlviii 217.
  1913d Concentration cells. JACS xxxv 344.
  1913e Concentration cells. AJP xxxi 343.
  1913f Concentration cells. TAES xxiii 401.
Bialaszwicz 1908 Swelling of egg. BASC 783.
  1912 Δ of chicks and tadpoles. AE xxxiv 489.
Bigelow 1898 Negative catalysers. ZPC li 585.
  1907 Filtration same through porcelain and colloidal membranes. JACS xxic 1675.
—— & Bartell 1909 Pores in membranes. JACS xxxi 1194.
Billárd & Bruyant 1905 Surface tension and locomotion. CRB lix 102.
—— & Dieulafe 1902 Surface tension of bile = .66. CRB liv 325.
Billiter 1913 Polyvalent ions and cataphoresis. APk (4) xi 902.
  1904 Clearing of sols with sols of same sign. BWA cxiii 1159.
  1905 Surface tension and electrode potential. ZPkC li 129.
Biltz, H. 1899 Phototropism of dyes. ZPkC xxx 527.
Biltz, W. 1904 Adsorption compounds of immune bodies. ZPkC xlviii 615.
  1904 Colloidal hydroxides positive. BDCG xxxvii 1095.
  1913 Osmotic pressure of dextrin. ZPkC lxxxiii 683.
—— & Steiner 1910 Adsorption hydrolysis. KZ vii 113.
Bingham & Durham 1911 Viscosity of suspensoid sols greater than water. ACJ xlvi 278.
Bjerrum 1908 KCl lowers diffusion potential. ZPkC liii 428.
Bock & Hoffmann 1871 NaCl infusion causes glycosuria. AP 550.
Bodenstein 1913 Photochemistry. ZPkC lxxxv 329.
—— & Dietz 1906 Enzyme changes equilibrium point. ZEC xii 605.
Bodländer & Fitting 1902 Solubility of ammoniacal silver salts. ZPkC xxxix 597.
Böhm 1909 Increase in blood pressure does not decrease volume. BZ xvi 313.
Bohn, G. 1904 Rhythm in littorina. CRA cxxxix 646.
  1907 Rhythm in diatoms. CRB lxii 121.
Bohr 1894 Secretion of gas into air bladder. JP xv 494.
  1904 Dissociation of oxyhemoglobin. ZP xvii 683.
—— & Hasselbach 1903 Calorimetry of embryos. SAP xxii 221.
Boldyreff 1915 Regurgitation from duodenum to lower gastric acidity. QJEP viii 1.
Bonnevie 1910 Mitosis. AZ v 1.
Boothby 1913 Anaesthesia. JPET v 379.
—— & Peabody 1914 Alveolar air. AIM 497.
Bordet 1899 Agglutinins do not agglutinate bacteria if ions are absent. AIP xiii 225.
Boruttau 1916 Ions and action current. ZP xxxi 1.
Bose 1913a Velocity of excitation impulse in plants. PT (B) cciv 63.
  1913b "Researches on the Irritability of Plants." Longmans.
Botazzi 1897 Δ of marine animals. AIB xxviii 61.
  1905 Δ of marine animals. AF iii 416.
  1909 Change of surface tensions of colloids. AF vii 593.
  1911 Surface tension of lymph = .89. Neuberg's "Die Harn."
  1913 Hemoglobin negative. AF xi 397.
—— & Craifaleanu 1916 PH of nerve juice = 6.04, of grey matter juice = 6.12. RRAL xxv 73.

—— & Ducceschi 1896 Δ and buffer value of blood. AIB xxvi 161.
—— & Enriquez 1901 Octopus gland in sea water absorbs water on stimulation. AIB xxxv 169.
Bournat 1909 Effect of ions on electroendosmose. CRA cxlix 1366.
Bousfield 1912 Ionic size. PRS (a) lxxxviii 147.
—— & Lowry 1910 Association of H_2O. TFS vi 15.
Boveri 1908 Viscosity of blood. PrM lxiii 546.
Bovie 1913 Coagulation by ultra violet light. S xxxvii 24.
1915 Direct reading potentiometer. JMR xxxiii 295.
Bradley 1910 Lipase. JBC viii 251.
1913 Enzyme synthesis. JBC xiii 407-431.
—— & Taylor 1916 Gelatine abolishes latent period in autolysis. JBC xxv 363.
Bredig 1894 Speed of organic ions. ZPkC xiii 191.
1898 Metal sols. ZEC iv 514, 547.
1899 Acids and alkalis increase dissociation of ampholytes. ZEC vi 33.
1901 "Anorganische Fermente." Leipsic.
1907 Specific catalytic action of potassium bichromate. Rhythmic catalysis. BZ vi 283.
—— & Fajans 1908 Asymmetric catalysis. BDCG xli 752.
—— & Fiske 1912 Asymmetry and catalysis. BZ xlvi 7.
—— & Weinmayr 1903 Rhythmic catalysis. ZPkC xlii 602.
Brodie & Vogt 1910 Increased respiration of gut during resorption. JP xl 135.
Brown, A. 1909 Permeability of barley grain same as plasma membrane. PRS (B) lxxxi 82.
Brown, H. 1914 Storage of oxygen by yeast. AnB xxviii 197.
Brown, O. 1903 Fundulus egg protoplasm anodic. AJP ix 111.
1905 Fundulus egg good insulator. AJP xiv 354.
Brown, W. 1915 Dilute alcohol increases permeability of collodion sacs. BJ ix 591.
Brünings 1903 Bioelectric currents. AGP c 367.
1907 Bioelectric currents. AGP cxvii 409.
Bürker 1910 Anodic oxidation of anaesthetic proportional to anaesthetic power. MMW lvii 1443.
Buchner & Rapp 1899 Fermentation and oxidation. ZB xxxvii 82.
Bugarsky 1897 Calculation of SO_4'' from electrode potential. ZAC xiv 145.
—— & Liebermann 1898 Buffer value of proteins. AGP lxxii 51.
—— & Tangl 1898 Conductivity of serum = .125 n NaCl. AGP lxxii 545.
Buglia 1907 Δ of blood increased by passage through active muscle. BZ vi 158.
1908 Maximum surface tension of emulsoids at isoelectric point. BZ xi 311.
Bunsen & Roscoe 1862 Photochemical law. APk cxvii 538.
Bunzel 1914 Oxidase apparatus. JBC.
1916 Oxidases. JBC xxiv 91, 103.
Burch 1892 Calibration of rapid excursion of capillary electrometer. PT (A) clxxxiii 81.
Burdon-Sanderson 1882 Action current of plants. PT 1.
1888 Action current of plants. PT clxxix (B) 417.
Burge 1914 Oxidation of pepsin. AJP xxxiv 140.
Burian 1910a Bony fish secrete hypotonic urine. AGP cxxxvi 741.
1910b Ultrafiltration. ZP xxiii 767.
—— & Drucker 1910 Small thermometer for Δ. ZP xxiii 772.
Burri & Nussbaum 1909 Surface tension of milk = .685. BZ xxii 90.
Burton 1906 Speed of colloids in potential gradient same as ions. PM (6) xi 425.

1906 Electrolytes decrease speed of colloid particles. Effect of polyvalent ions. PM (6) xii 472.
Burton-Opitz 1911 Viscosity of blood. JAMA lvii 353.
1914 On laking blood becomes more then less viscous. AJP xxxv 51.
Busquet & Pachon 1908 Ca on heart. CRB lxv 599.
Buxton & Rahe 1909 Opposite sols precipitate only in equivalent proportions. JMR xx 113.
—— & Shaffer 1906 Electrolytic solution tension and precipitating power. ZPkC lvii 47.
——, Shaffer & Teague 1906 Reversal of charge of sols with sols of opposite sign. ZPkC lvii 47, 64.
Byers & Walter 1914 Electrolytic endosmose. JACS xxxvi 2284.
Callaud (& Pelletier) 1821 Chemiluminescence. JPm vii 579.
Calugareanu 1910 Anaesthetics increase size of colloid particles by dissolving in them. BZ xxix 96.
—— & Henri 1902 Salts diffuse from erythrocytes into isotonic sugar solution. CRB liv 210, 356.
Campbell, Douglas, Haldane & Hobson 1913 CO_2, H_2 and respiratory center. JP xlvi 301.
Cannon 1897 Movements of stomach. AJP i 359.
—— & Cattell 1916 Action current of glands. AJP xl 143.
Carlson 1906 Anesthetics increase rhythm. AJP xvii 182.
1911 Stretching nerve and conduction rate. AJP xxviii 323.
——, Greer & Luckhardt 1908 Δ of lymph and blood same yet lymph contains more colloids. AJP xxii 91.
——, Orr & Brinkman 1914 Powlow-gastric juice. AJP xxxiii 86.
Carrell & Guthrie 1905 Transplanted kidney secretes hypotonic urine. S xxii 563.
Cary 1916 Regeneration. JEZ xxi 1.
Cathcart 1904 Serum antitryptic. JP xxxi 497.
Cernovodeanu & Henri 1906 Bacteria are anodic. CRB lxi 200.
Cesana 1913 Ultramicroscopy of catalysis. AF xi 130.
Chambers 1915 Microdissection. S xli 290.
Chapin 1902 CO_2 stimulates plant growth similar to ether. F xci 348.
Chiari 1909 Ether increases antolysis. AEP lx 256.
1911 Action of acids and alkalis on swelling of glutin. BZ xxxiii 167.
—— & Fröhlich 1911 Vagus action on heart abolished with oxalates. AEP lxvi 110.
—— & Januschke 1911 Formation of exudates inhibited by Ca. AEP lx 120, WKW xxiii 427.
Chick & Martin 1912 Ppt. of heated proteins. JP xlv 261.
Child 1911 Amitosis. BB xxi 280.
1913 Cell respiration. JEZ xiv 153.
Choquard 1913 Narcosis of fat and lean tissues. ZB lx 1.
Christianson, Douglas & Haldane 1913 CO_2 from blood. JP xlvii (p ii).
Clark 1899 In prolonged action of Hg salts ionization unimportant. JPC iii 263.
Clark, A. 1913 Action of ions and lipoids on frog's heart. JP xlvii 66.
Clark, W. 1915a PH of cultures. JBC xxii 87.
1915b H_2 electrode. JBC xxiii 475.
1915c PH of modified milk. JMR xxxi 431.
1915d PH of cultures. JID xvii 109.
—— & Lubs 1915 PH of cultures. JID xvii 160.
1916 Buffer mixtures. JBC xxv 479.
Clarke 1911 Analysis of sea water. BGS (no. 491) 113.
1916 Ash of marine invertebrates. S xliii 723.
Clausen 1890 Respiration of plants doubled by 10° rise in temperature. LJ xix 893.

Clowes 1903 Δ and sp. gr. of urine. AJP ix 319.
1916 Soap gelation and permeability. PSEB xiii 114, JPC xx 407.
Coblentz 1912 Temperature and spectrum of firefly. CIWP (No. 164).
Coehn 1898 Suspensions of lower dielectric constant than water are anodic. WAP lxiv 217.
1909 Tyndall phenomenon of sugar solutions. ZEC xv 652.
—— & Barrat 1905 Galvanotropism. ZAP v 1.
Cohnheim 1898 Very hypertonic solution in gut draws water and salts out of blood. ZB xxxvi 129.
1901 Negative osmose through holothurian gut. ZPC xxxiii 9.
1902 Negative osmose through gut of octopus. ZPC xxxv 416.
1913 Chopped kidney binds NaCl and sugar when it exceeds a certain concentration. ZPC lxxxiv 451.
—— & Von Uexknell 1912 Catch-action of smooth muscle. ZPC lxxvi 314.
Colton 1910 Surface tension. PANS 42.
Compton 1915 Optimum PH for maltase 7.2 at 47°, 3 at 35°. PRS (B) lxxxviii 408.
Conklin 1908 Movements in dividing eggs. Biol. Lect. Woods Hole. 69.
1912 Cytoplasm nucleus ratio. JEZ xii 1.
Constantino 1914 Erythrocytes permeable to amino acids. AIB lx 442.
Cooke 1898 Increased osmotic pressure of tetanized muscle. JP xx 137.
Corral 1915 Dilution to ½ does not change PH of blood. BZ lxxii 1.
Correns 1891 Turgor. JWB xxii 161.
Craw 1905 Toxin-antitoxin reaction. PRS (B) lxxvi 179.
Cramer 1915 Surface tension and metabolism. PRS (B) lxxxviii 584.
Cremer 1906 Concentration cells with membranes permeable only to H ions. ZB xlvii 562.
Cumming & Gilchrist 1913 Diffusion potential in wide tube. TFS ix 174.
Curtis, H. A. —— Non-inductive resistances. BBS (no. 3, 8).
Cushny 1901 Diuresis and permeability. JP xxvii 429.
1902 Blood pressure and diuresis. JP xxviii 443.
Cybulski 1903 Membranes and concentration cells. BASC 622.
—— & Dunin-Borokowski 1909 Effect of gelatine membrane on concentration cell. BASC 660.
Czapek 1902 Homogentisinic acid and geotropism. BDBG xx 464.
1910 Plants killed by solutions of surface tension = .68. BDBG xxviii 159, 490.
Dahlgren 1914 Origin of electric organ. CIWP (No. 183) 159.
Dakin, H. 1905 Asymmetry and enzyme action. JP xxxii 199.
1912 "Oxidations and Reductions in the Animal Body." Longmans.
Dakin, W. 1908 Change of Δ of fishes. BJ iii 258.
Dale 1911 Galvanotropism of infusoria. JP xxvi 291.
Danysz 1902 If antitoxin is added slowly more free toxin is left than if added suddenly. AIP xvi 331.
D'Arsonval 1889 Surface tension and bioelectric phenomena. AP 460.
1901 Resistance of bacteria to cold. CRA cxxxiii 84.
Darwin, Francis 1903 Artificial rhythm in plants.
Davenport 1897 Swelling of frog's embryos. PBS xxviii 73.
Davidsohn 1912 Proteolysis in infants' stomachs. ZK iv 208.
1913a Methods for gastric analysis. Buffer value of stomach. ZK ix 470.
1913b PH and lipase. BZ xlviii 249.
Davies 1907 Adsorption and solid solution. JCS ix 1666.
Davis 1915 Surface tension of H_2SO_4 solutions. Thesis, Columbia U.
Dawes 1905 Adsorption of colloids irreversible. BCPP vi 426.
Deetjen 1909 Alkalinity causes dissolution of blood platelets. ZPC lxiii 1.
Dekhuisen 1904 Δ of fresh water animals. ANd x 121.
Delage 1902 CO_2 causes parthenogenesis of starfish. AZEG (3) x 213.
1907 Parthenogenesis. AZEG xxxvi (N & R no. 2).

DeMeyer 1911 Swelling of sperm in egg extracts. AB xxvi 65.
Demoor 1894 Asphyxia of cells. AB xiii 163.
1907 Swelling and Δ of organs. AIP iv 340.
D'Errico 1907 Δ of fowl = .616°. AF ix 453.
Denham 1908 Salt hydrolysis. TCS xciii 41.
Denis 1913 Fish blood and urine. JBC xvi 389.
DeVries 1884 Δ of plant juices. Isotonic solutions. JWB xiv 427.
1885 Permeability of vacuole membrane. JWB xvi 465.
Dietz 1907 Catalysis in diphasic system. ZPC lii 279.
Dole 1914 Analysis of sea water. CIWP (no. 182) 71.
Donaldson 1916 "The White Rat."
Donders 1872 Dissociation of oxyhemoglobin. AGP v 20.
Donnan 1911 Osmotic pressure of Congo red. ZEC xvii 572.
—— & Donnan 1905 Surface tension of urine, normal = .9, icterus = .75. BMJ 1636.
—— & Harris 1911 Osmotic pressure of Congo red. TCS xcix 1554.
—— & Potts 1910 Dispersion of soaps. Haptogen membranes and emulsions. KZ vii 208.
Douglass, Haldane & Haldane 1912 Dissociation of oxyhemoglobin. JP xliv 275.
——, Haldane, Henderson & Schneider 1913 Adaptation to high altitudes. PT (B) cciii 185.
Dreser 1893 Toxicity and ionization. AEP xxxii 456.
Drew 1914 Denitrifying bacteria in sea water. CIWP (no. 182) 9.
Dreyer & Roy 1911 Blood volume. PT (B) ccii 191.
—— & Walker 1912 Resistance of dry bacteria to heat. JPB xvii 142.
Drury 1914 Microchemistry of oxidation. PRZ (B) lxxxviii 166.
Drucker 1913 Calculation of Ba$^{\cdot\cdot}$ from electrode potential. ZEC xix 804.
Dubois 1913 "Mechanisme intime de la production de la lumiere chey les organismes vivants" (Soc. Linneenn de Lyon) (A. Rey).
DuBois-Reymond Resistance of muscle less in tetanus (II, i, 82). "Untersuchungen ueber tierische Elektricitaet."
1916 Muscle contraction. BKW liii 393.
Duclaux 1870 Surface tension curve. ACP (4) xxi 383, 427.
1905 Osmotic pressure of suspensoids. CRA cxl 1544.
1909 Osmotic pressure of colloids. JCP vii 405.
Dupré 1866 Surface tension-adsorption curve. ACP (4) vii 409, ix 379.
Durig 1901 Absorption of water by frogs. AGP lxxxv 401.
Dutrochet 1835 Negative osmose. ACP (2) lx 337.
Ebbecke 1894 Sudden increase of pressure to 300 atmospheres causes contraction of narcotized muscle. AGP clvii 79.
Edridge-Green 1915 Color vision. JP xlix 265.
Ehrenberg 1913 Lyotrope series in gelatine swelling depends upon concentration of salts. BZ liii 356.
Ehrlich 1885 "Das Sauerstoff-Bedürfniss des Organismus." Berlin, Hirschwald.
Einstein 1908 Brownian movement. ZEC xiv 235.
Einthoven 1901 String galvanometer. ANd (2) vi 625.
Eisenberg & Okoloska 1913 NaCl increases toxicity of phenol. ZB lxix 312.
—— & Volka 1902 Adsorption of agglutinin by bacteria. ZH xl 154.
Eisler 1909 Pure salt solution cause involution forms of bacteria. ZB (1) li 546.
—— & Von Portheim 1909 Al$^{\cdot\cdot\cdot}$ retards entrance of methyl violet into cells. BZ xxi 59.
Ellis, J. 1916 Concentration (activity) of H$^{\cdot}$ in HCl. JACS xxxviii 737.
Ellis, R. 1912 Effect of ions on oil sols. ZPkC lxxx 597 and lxxviii 321.
Embden & Kraus 1912 Lactic acid produced by liver. BZ xlv 1.
—— & Kondo 1912 Lactic acid production in muscle-press-juice inhibited by H$^{\cdot}$ (addition of alkali increases yield). BZ xlv 63.

Emmerling 1901 Specificity of enzymes. BDCG xxxiv 600, 2206, 3810.
Emslander 1914 PH of liver. KZ xiv 44, xxiv 3810.
Endler 1912 Permeability of protoplasm. BZ xlii 440, xlv 402.
Engelmann 1882 Phototropism. AGP xxix 387.
1893 Violin E-string swells and shortens in dilute acids or alkalis or on heating. "Ueber den Ursprung der Muslelkraft." Leipsic.
Engler & Wild 1897 Peroxides and oxidation. BDCG xxx 1669.
Erlenmeyer 1913 Asymmetry and synthesis. BZ lxiv 382.
Euler 1907 Displacement of equilibrium point by enzymes. ZPC lii 146.
—— & Pope 1912 "General Chemistry of Enzymes." N. Y. Wiley.
Erb 1906 Adrenalin causes reduction of blood volume. DAKM lxxxviii 36.
Erlanger & Garrey 1914 Faradic stimuli. AJP xxv 337.
Ernst 1901 Temperature optimum for catalysis. ZPkC xxxvii 448.
Evans 1906 Adsorption of NaCl, KCl, etc. JPC x 290.
Evans & Schuleman 1914 Permeability to colloidal dyes. S xxxix 443.
Ewald 1912 Reversal of phototropism by electrolytes. JEZ xiii 591.
Ewart 1903 "Protoplasmic Streaming in Plants." Oxford, Clarendon Press.
Fahr 1909 Permeability of muscle. ZB lii (NF) xxxiv 72.
Fajans 1910 Asymmetry and catalysis. ZPC lxxiii 25, lxxv 232.
Falk & Nelson 1912 Hydrolytic action of amino acids. JACS xxxiv 828.
Fallois 1901 Increased oxygen pressure. AB xvii 713.
Fano & Botazzi 1896 Δ of portal blood. AIB xxvi 45.
—— & Mayer 1907 Surface tension of serum = .82. AF iv 165.
Faraday 1834 Catalysis by metals. PT.
1857 Protection colloids. PT 154.
Farkas 1903 PH of serum. AGP xcviii 551.
—— & Scipiades 1903 Blood during pregnancy. AGP 577, 581.
Fenton 1894 Oxidation of tartaric acid by iron. TCS lxv 899.
Fernbach 1890 Alcohol increased permeability of fungi to enzymes. AIPr iv 672.
1906 PH and diastase. CRA cxlii 285.
Field & Teague 1907 Immune bodies cathodic. JEM ix 86.
Fienga 1910 Effect of salts on smooth muscle. ZB liv 230.
Findlay 1908 Adsorption of CO_2 by sols. KZ iii 169.
1913 "Osmotic Pressure." Longmans.
Fischel 1906 Granules and cell division. AE xxii.
Fischer, A. 1895 Permeability of bacteria to salts.
1899 "Fixierung Färbung and Bau des Protoplasmas." Jena.
Fischer, E. 1898 Asymmetry and enzyme action. ZPC xxvi 60.
—— & Abderhalden 1907 Asymmetry and enzyme action. ZPC li 264.
Fischer, H. & Jensen 1909 Freezing out of colloids. BZ xx 143.
Fischer, M. 1908 Salts reduce the swelling action of acids and bases on proteins. AGP cxxv 99.
1914 "Oedema and Nephritis." 2nd Ed. N. Y.
—— & Moore 1907 Action of acids and alkalis on swelling of fibrin. AJP xx 330.
—— & Sykes 1914 Alcohol and acetone retard swelling of gels. KZ xiv.
Fitting 1906 "Reizleitungsvorgänge bei der Pflanzen." EP v 155.
1911 Δ of desert plants. ZB iii 209.
Fitzgerald 1910 Location of HCl in gastric tubules. PRS (B) lxxxiii 56.
Fleisher & Loeb, L. 1910 Absorption from serous cavities. JEM xii 288, 487.
Fleischhamer 1913 Salts produce current of injury of heart. ZB lxi 326.
Fletcher 1913 Lactic acid in muscle. JP xlvii 361.
—— & Brown 1914 CO_2 production and beat rigor of muscle. JP xlviii 177.
—— & Hopkins 1907 Lactic acid is not burned by O_2 if muscle is chopped up. JP xxxv 247.
Flexner & Noguchi 1906 Diffusion of colloids. JEM viii 547.

Fluri 1909 Polyvalent ions increase permeability of Spirogyra. F xcix 81.

Flusin 1908a Negative osmose and swelling of membrane. ACP (8) xiii 480.

1908b Osmose and hydration of membrane. JPq (4) vii 291.

Forschhammer 1865 Analysis of sea water. PT clv 203.

Forster 1873 Dogs die from lack of salt. ZB ix 297, 369.

Fowler, Bergeim & Hawk 1915 Indicators for stomach. PSEB xiii 58.

Fraenckel 1904 Curve of conductivity of blood, and erythrocyte volume. ZKM lii (h5).

1905 PH of gastric juice. ZEP i 1, 431.

1907 Catalyser used up in catalysis. ZPC lx 202.

Frankforter & Kritchensky 1914 Catalysis. UMS (No. 2).

Franz, F. 1903 Hirudin. AEP xliv 342.

Franz, V. 1909 Δ of marine animals. IRGH ii 557.

Fredericq 1904 Δ of shark changes with sea water. AB xx 709.

Frenkel & Cluzet 1901 Surface tension of urine. JPP iii 99.

Freund 1913 Na favors and Ca inhibits fever. AEP lxxiv 311.

Freundlich 1903 Rate of addition of electrolyte to sol. ZPkC xliv 143.

1907 Adsorption: formula, reduction of heavy metals by. ZPkC lvii 385.

1909 "Kapillarchemie." Leipsic, Akad Verlag.

1910 Adsorption, solid solution, potential and precipitating power. ZPkC lxxiii 385, 397.

1916 Negative osmose. Electrostenolysis. KZ xviii 11.

—— & Bjercke 1916 Oxidation rate of oxalic acid by charcoal determined by diffusion. ZPkC xci 31.

—— & Loser 1907 Adsorption hydrolysis. ZPkC lix 284.

—— & Mäkelt 1909 E M F produced by gravity or suspensions. ZEC xv 161.

—— & Neumann 1909 Ionic exchange. Reversibility of adsorption. ZPkC lxvii 538.

—— & Schucht 1912 Precipitation of As_2S_3-sol by ions. ZPkC lxxx 564.

1913 Adsorption and suspensoid precipitation. ZPkC lxxxv 641.

—— & Von Elissafoff 1912 Adsorption potential of colloids. ZPkC lxxix 407.

Frey 1910 Reduction of Cl in blood by feeding NaBr. ZEP viii.

1912 Narcosis with ethyl chloride. BZ xl 29.

Friedemann & Friedenthal 1906 Albumins precipitated by nucleic acid. ZEP iii 73.

Friedenthal 1900 Proteins and carbohydrates not absorbed from gut by lacteals. AP 252.

1903a Buffer value of serum. AP (physiol. Gesell. 1903).

1903b Indicators. ZEC x 113.

1903c Tensimeter. ZP xvii 437.

1904 PH of serum and indicators. ZAP iv 44.

1911 Significance of salts in milk. MMW 2385.

Frölich & Meyer 1912 Action-current of contracted muscle. ZP xxvi 269.

Frozee 1909 Electrical stimulation to growth. JEZ vii.

Fühner 1912 Narcosis and evolution. ZB lvii 465.

—— & Neubaur 1907 Hemolysis, $H^{\cdot}$ and OH', and partition coefficient. AEP lvi 333.

Gager 1908 Radium and growth. AN xlii 761.

Galeotti 1901 $Na^{\cdot}$ antitoxic to Cu sol. BC xxi 321.

1904 Electrodes for conductivity of tissue. ZB (nf) xxv 289.

1905 Water content of emulsoid particles. ZPC xliv 461.

1906 Bioelectric currents. ZAP vi 99.

Gallardo 1906 Model of mitosis. CRA cxlii 228.

Ganter 1912 Temperature coefficients. AGP cxlvi 185.

Gardiner, W. 1884 Pores in plant cell walls. PT clxxiv 817.
1897 Surface movements of dividing eggs. Jour. of Morph. xi 55.
Garmus 1912 Increased permeability of gland cells during activity. ZB lviii 185.
Garten 1906 Time of appearance of current of injury. ZP 673.
1909 Rhythmic response with single stimulus. ZB lii 534.
1911 Electric organ. VGDN 1.
Garrey 1913 Resistance of fish to change in medium. AJP xxxix 313.
1915 Δ of animals and solutions. BB xxviii 77.
Gaskell 1886 Positive variation of heart during vagus inhibition. JP vii 451.
Gassner 1906 Pos. and Neg. galvanopropism of roots. BZ lxiv 149.
Gasser & Loevenhart 1914 Respiratory center and asphyxia. JPET v 239.
Gatin-Gruzewska 1904 Δ of glycogen = 0. AGP ciii 282.
—— & Biltz 1904 Ultramicroscopy of albumin. AGP cv 115.
Gerassimow 1902 Cell nucleus ratio. ZAP i 220.
Ghiron 1913 Dyes filter through glomeruli. AGP cl 405.
Gibbs 1874-8 Surface tension and adsorption. TCA iii 380.
Gibson & Titherly 1908 Photoelectricity and photosynthesis. AB xxii 117.
Gildemeister 1913 Polarizability of skin and psychogalvanic reflex. MMW 2389.
1915 Psychogalvanic reflex. AGP clxii 489.
Girard 1908-13 Negative osmose and electroendosmose. CRA cxlvi 927; cxlviii 1047, 1186; cl 1446; cliii 401; clvi 1401.
1910 Negative osmose and turgor. JPP xii 471.
1913 Hemolysis and electroendosmose. CRB lxxiv 520.
Glaser 1912 Calorimetry of embryos. S xxxv 189.
1913 Parthenogenesis. S xxxviii 446.
1914a Parthenogenesis. BB xxvi 387.
1914b Shrinkage of egg on fertilization. BB xxvi 84.
1915 Fertilization. BB xxviii 149.
Göthlin 1902 Saline for frog's heart. SAP xii 1.
Goldschmidt 1899 Catalytic reaction in diphasic system. ZPkC xxxi 235.
—— & Pribram 1909 Lipoids precipitated by anaesthetics. ZET vi 1.
Goodridge & Gies 1911 Edema. SEBM viii (106).
Gortner & Harris 1913 Δ of plant juices. BTBC xl 27.
1914 Cryoscopic method. PW xvii 49.
Gotch & Laws 1885 Limulus blood ash. 54 Meeting Brit. Assn. 774.
Gouy 1906 Lyotrope series and surface tension. ACP (8) ix 75.
Graham 1854 Negative osmose of acids reduced by salts. PT cxliv 177.
1861 Colloids and rate of diffusion. PT cli 183.
Gray 1913a Increased conductivity of egg. PCPS xvii 1.
1913b Increased conductivity of egg. JMBA x 50.
1915 Agglutination of sperm with ions. QJMS lxi 119.
Greely 1904 Galvanotropism. JP vii 3.
Greene 1904 Δ of salmon. BBF xxiv 429.
1909 Δ of salmon. BBF xxix 129.
1910 Δ of salmon. JEZ ix.
Griffiths 1892 "Physiology of Invertebrata." London.
Grijns 1896 Hematocrit and osmotic pressure. AGP lxiii 86.
Grode & Lesser 1913 Crushing liver greatly increases splitting of glycogen. ZB lx 371.
Gros 1910 Adsorption of NH_3 and hemolysis. BZ xxix 350.
1910 Neuraxons require 6 times as much anaesthetic as for general anaesthesia. AEP lxii 379.
1912 Local anaesthetics are effective on all cells. AEP lxvii 132.
Grünwald 1909 Chloride starvation has same effect on nervous system as bromide poisoning. AEP lx 360.

Grützner 1893 Myelin sheath inhibits diffusion. AGP liii 115.
1894 Lyotrope series and excitability of nerve. AGP lviii 69.
Grumbach 1911 Anesthetics and contact potentials. ACP xxiv 463.
Guldberg & Waage 1879 Law of mass action. ZPC cxxvii 69.
Gudzent 1909a Dissociation and isomerism of uric acid. ZPC lx 25, 38; lxiii 253.
1909b Diffusible uric acid in serum. ZPC lxiii 466.
1913 Colloidal solutions of uric acid. ZPC lxxxix 253.
Guthrie 1914 Hypertonic laking. PSEB xi 149.
—— & Ryan 1910 Action of Mg. AJP xxvi 329.
Guye 1910 Association of H_2O. TFS vi 8.
Haber 1908 Solution tension and polarization. APk (4) xxvi 927.
—— & Klemensiewicz 1909 Concentration cells with membranes permeable only to H ions. ZPkC lxvii 385.
Haberlandt 1890 Geotropism of plants. BDBG xviii 261.
Hägglund 1915 Fatty acids inhibit yeast more than calculated from PH. BZ lxix 181.
Hagan & Ormond 1912 Vagus action on heart abolished with pure NaCl solution. AJP xxx 105.
Haldane 1892 Blood-gas apparatus. JP xviii 419.
—— & Priestly 1905 .2% rise in alveolar CO_2 doubles lung ventilation. JP xxxii 225.
Halliburton 1916 Physiological Abstracts. London. i—.
Hallwachs 1888 Photoelectricity. APk xxxiii 301.
Hamburger 1890 Plasmolysis. ZPC vi 311.
1892 CO_2 causes Cl to pass from plasma into corpuscle. ZB xxviii 405.
1893 Hematocrit and osmotic pressure. ZP.
1896 Absorption from peritoneum increased by pressure. AAP 302.
1898a Hemolysis with acids. AAP xxxi.
1898b Diffusible and non-diffusible alkali in serum. AB 1.
1899 Protoplasm not a solution. AAP.
1902 "Osmotischer Druck und Ionenlehre in der Med. Wiss." Wiesbaden.
1908 Negative osmose through double membrane. BZ xi 443.
1912 "Untersuchungen ueber Phagocyten." Wiesbaden. Bergmann.
1915 Micro-analysis of K. BZ lxxi 415.
1915 Phagocytosis. IZPB ii 245, 249, 255.
1916a Ca, anesthetics, CO_2 and lack of oxygen increase phagocytosis. BMJ 37.
1916b Erythrocytes permeable to Na and K. WMM lxvi 521, 575.
—— & de Haan 1910 Effect of salts on phagocytosis. BZ xxiv 304.
1913 Solubility, surface tension, toxicity and narcotic power. AAP 77.
—— & Hekman 1908 Phagocytosis. BZ ix 275.
—— & Van Lier 1902 CO_2 causes NO'_3 and SO''_4 to pass into erythrocyte. AAP 492.
Hamill 1906 Antitrypsin. JP xxxiii 476.
Hammersten, Hedin & Mandel 1914 "Text Book of Physiological Chemistry." 7th Ed. N. Y.
Handowsky 1910 Hydrolysis of alkali albuminate. BZ xxv 510.
1912 Partial hemolysis. AEP lxix 412.
Harden & McLean 1911 Cell oxidation. JP xliii 417.
Hardy 1899 Structure of emulsoid coagulum and protoplasm. Precipitation of sols with $H^{\cdot}$. JP xxiv 158, 301.
1900a Solution. JPC iv 235, 254.
1900b Isoelectric point = least stability, and precipitation by opposite charge. ZPkC xxxiii 385.
1905 Isoelectric point of proteins. JP xxxiii 251.
—— & Whitham 1899 Coagulation of protein by electricity. JP xxiv 288.

Harkins & Humphrey 1916 Stalagmometer corrections. JACS.
Harlow & Stiles 1909 Shaking enzymes. JBC vi.
Harris 1914 Acid soils due to negative colloids. JPC xviii 355.
Harris, J. & Gortner 1914a Conversion table for Δ. AJB i 75.
  1914b Conversion table for Δ. BCB iii 259.
  1915a Δ and conductivity of plant juices. BCB iii 196.
  1915b Δ and conductivity of plant juices. BCB iv 52.
Harrison 1910 Neuraxon growth and ameboid motion. JEZ ix 787.
Hartridge 1912 Color vision. JP xlv (p. xxix).
Harvey 1910a Fert. Mem. imperm. to sugar and proteins. JEZ viii 355.
  1910b Parthenogenesis (Review). BB xviii 269.
  1911 Cell permeability. JEZ x 508.
  1912 Permeability of artificial cells. BCB ii 50.
  1913 Indicator method and permeability. AJP xxxi 335.
  1914a Fertilization membrane. BB xxvi 237.
  1914b Permeability to acids. IZPB i 463.
  1914c Permeability to alkali. CIWP (no. 183) 131.
  1915a Photogenesis. JACS xxxvii 396.
  1915b Photogenesis. AJP xxxvi 230.
  1916 Photogenesis. S xliv 208; 652.
Hasselbalch 1911 H_2-electrode. BZ xxx 317.
  1912 PH and respiratory center. BZ xlvi 413.
  1913 Improved H_2-electrode. PH .17 lower at 18.9° than at 37°. BZ xlix 447, 451.
—— & Gammeltoft 1915 PH of blood. BZ lxviii 206.
—— & Lundsgaard 1912 PH of blood. BZ xxxviii 77.
Hatschek 1910 Ultrafiltration. KZ vii 81.
  1911 Viscosity of emulsoids due to swelling. KZ viii 34.
  1913 Volume of disperse phase in emulsions. KZ xii 238.
Hauberisser & Schoenfeld 1913 Effect of salts on swelling of tendons. AEP lxxi 102.
Haycraft 1883 Hirudin. AEP xviii 209.
Heald 1896 Electrolytic solution tension and toxicity. BZ xxii 125.
Hedin 1891 Hematocrit. SAP ii 134.
  1895 Hematocrit and osmotic pressure. AGP lx 360.
  1895 Hematocrit and osmotic pressure. AGP lx 360.
  1898 Permeability of erythrocytes. AGP lxx 525.
  1899 Rate of diffusion through dead gut. AGP lxxviii 205.
  1906 Enzyme action inhibited by adsorption of enzyme. BJ i 484.
  1909 Adsorption of colloids irreversible. ZPC lx 364.
Heidenhain 1872 Blood pressure and formation of lymph. AGP v 309.
  1894 Negative osmose through gut wall. AGP lvi 579.
Heilbrunn 1913 Membrane elevation due to decreased surface tension. BB xxiv 343.
  1915a Development and gelatin of egg. BB xxix 149.
  1915b Oxidation in egg. S xlii 615.
Heimrod 1913 Supposed catalytic action of oscillating magnetic field. ZAC xix 812.
Hekma 1915 Blood coagulation. IZPB ii 279, 299, 352.
Heller 1911 Δ of blood rapidly increases after nephrectomy even in starving rabbit. ZP xxv 375.
Hemptinne 1898 H in atomic form in Pd. ZPkC xxvii 429.
Henderson 1907 Formula for diffusion potential. ZPkC lix 118.
Henderson, L. 1908 Buffers. AJP xxi 173.
  1909 Dissociation of CO_2. EP viii 254, 274, 316.
  1910 PH of urine. BZ xxiv 40.
  1911 Kidneys regulate PH of blood. JBC ix 403.
  1913 "The Fitness of Environment." Macmillan.

—— & Bloch 1908 Buffer value of CO_2. AJP xxi 420.
—— & Spiro 1908 Buffer value of urine. BZ xv 105.
Henderson, V. 1910 Antitoxic action of sugar. ZP xxiv 519.
Henderson, Y., Palmer & Newburgh $H^{\cdot}$ and colloid swelling. JPET v 449.
Henri 1904 Erythrocytes anodic. CRB lvi 866.
1911 Photochemistry of sight. JPP xiii 841.
—— & Lalou 1903 Δ of shark changes with sea water. CRA cxxxvii 721.
—— & Mayer 1904 Adsorption potential. CRA cxxix 974.
Henze 1910 Increased O_2 increases respiration of coelenterates. BZ xxvi 255.
1913 Blood corpuscles of acidians are acid. ZPC lxxxvi 340.
Herbst 1893 Artificial production of fertilization membrane. BZ xiii 14.
1898 OH' necessary for egg development. AE vii 486.
1904a Na, K, Ca, Mg, Cl, SO_4, HCO_3, and OH necessary development of sea urchin egg. AE xvii 306.
1904b Ca-free water causes cells to fall apart. AE xvii 440.
Herlitzka 1912 Addition of urea, glycerine, arctameide, chloral hydrate or urethane improves Ringer's fluid. AF x 261.
Hering 1915 KCl stimulates vagus center. AGP clxi 537.
Herzog 1902 Zymase action is autocatalytic. ZPC xxxvii 149.
—— & Betzel 1911 $CHCl_3$ and $AgNO_3$ adsorbed by yeast but formaldehyde chemically combined. ZPC lxxiv 221.
—— & Kasarnowski 1908 Crystalloids diffuse more than 19 times as fast as colloids. BZ xi 172.
—— & Meier 1909 Asymmetry and enzyme action. ZPC lix 57.
1911 Oxydases. ZPC lxxiii 258.
Hess 1913 Infant gastric juice. AJDC vi 264.
Heubner 1905 Viscosity of blood. AEP liii 280.
Heymans 1912 Permeability of filter ultrafilter and membranes to microbes. AIPd xxii 49.
Higgins 1913 CO_2 (Plesch, 20 sec.) 20% higher than Haldane. CIWP (No. 203) 171.
—— & Means 1915 Alv. CO_2 in acidosis. JPET vii 1.
Hill, A. 1898 Synthesis of isomaltose. JCS lxxiii 634.
1903 Enzymatic splitting and synthesis of sugars. JCS lxxxiii 578.
Hill, A. V. 1910 Formula for Nernst's theory of stimulation. JP xl 190.
1912 Nerve impulse produces no heat. JP xliii 433.
1913a Half of heat of muscle produced during contraction; half during restitution. JP xlvi 28, 435; xlvii 305.
1913b Dissociation of oxyhemoglobin. BJ vii 471.
1914 Oxidation of lactic acid. JP xlviii (p. x).
1915 Formula for blood-gas apparatus. JP l (vii).
—— & Weizsäcker 1914 Myothermic apparatus. JP xlviii (p. xxxv).
Hill, L. & Nabarro 1895 Not always more CO_2 in venous blood from active organ (vascular dilatation). JP xviii 220.
Hirokawa 1908 Swelling of pores of kidney due to urine. AF xi 458.
1909 Effect of salts on spermatozoa. BZ xix 291.
Hirschfeld 1907 $HgCl_2$ may lake erythrocytes. AH lxiii 275.
1909 Ameboid motion. ZAP ix 529.
Hirschfelder 1911 Electrocardiogram. Inters. Med. Jl. xviii (6).
His & Paul 1900 Dissociation of uric acid. ZPC xxxi 1, 64.
Höber 1899 Rate of diffusion and adsorption by gut. AGP lxxiv 225, 246.
1900 PH of blood. AGP lxxxi 522.
1903a Selective adsorption of iron by gut (other heavy metals absorbed less). AGP xciv 337.
1903b PH of blood. AGP xcix 572.
1904a Erythrocytes and yeast are anodic; changed by polyvalent ions, impermeable to $Na^{\cdot}$, $K^{\cdot}$, $NH_4^{\cdot}$, $Ca^{\cdot\cdot}$ and $Mg^{\cdot\cdot}$. AGP ci 607, cii 196.

1904b Δ of fresh water animals. AGP cii 199.
1905 Alkali salts increase permeability of muscle. AGP cvi 599.
1907 Current of injury reduced by anaesthetics but caused by toxic dose of anaesthetics. AGP cxx 492.
1907 Inverse lyotrope series for positive and negative proteins and irregular series for neutral proteins. Series for lecithin. BCPP xi 35.
1908a Hemolysis and lyotrope series. BZ xiv 209.
1908b Physikalische Chemie d. Blutes und. Lymphi. Oppenheimer's Handb. d. Biochem. II 2, 1.
1909a Theory of skin current. ZEC xv 519.
1909b Effect of salts on cilia. BZ xvii 518.
1909c Dispersion of dyes. BZ xx 56.
1909d Permeability of erythrocytes. ZPC lxx 134.
1910a Conductivity of cell interior. AGP cxxxiii 237.
1910b Effect of organic anions on cells. AGP cxxxiii 311.
1910c Alkali salts increase permeability of muscle. AGP cxxxiv 316.
1910d Stimulation. ZAP x (Ref.) 173.
1912a Lung is permeable to NH$_3$. AGP cxlix 87.
1912b Conductivity of cell interior. AGP cxlviii 189.
1913a Muscle contraction. ZEC xix 738.
1913b Increase of conductivity of blood with increased alternations of current. Cond. of interior of muscle fibre. AGP cl 15.
1913c Bleaching of wool-violet by protoplasm. ZB xlviii 291.
1914 "Physikalische chemie der Zelle und der Gewebe." 4th ed. Leipsic.
—— & Jankowsky 1903 PH of urine. BCPP iii 525.
—— & Kempner 1908 Kidney cells absorb all dyes but suspensoids. BZ xi 105.
—— & Königsberg 1905 Dyes excreted by frog's kidney carried in preformed granules. AGP cviii 323.
—— & Nast 1914 Saponin hemolysis and lyotrope series. BZ lx 131.
—— & Spaeth 1914 Rare earths on muscle. AGP clix 433.
—— & Waldenberg 1909 Effect of organic ions on cells. Position of Cs in lyotrope series. AGP cxxvi 331.
Hoffmann 1889 Buffer value of proteins. ZIM x 793, x 521.
1914 Haptogen membranes and emulsions. Collection of particles at surface. ZB lxiii 386.
Hofmeister 1888 Hofmeister series. AEP xxiv 247.
1891 Hofmeister series. AEP xxviii 210.
Hoitsema 1895 Hydrogen in atomic form in Pd. ZPkC xvii 1.
Hooker 1911 Ions and CO$_2$ on vascular tone. AJP xxviii 361.
1912 Effect of CO$_2$ and O$_2$ on vascular tone in the gut. AJP xxxi 47.
Howe & Hawk 1912 PH of feces. JBC xi 129.
Howell 1914 Blood coag. AJP xxxv 143, AIM xiii 76.
—— & Duke 1908 Vagus inhibition and KCl. AJP xxi 51.
Howland & Marriott 1916a Acidosis and diarrhea. AJDC xi 309.
1916b Alv. CO$_2$ and acidosis. JHHB xxvii 63.
Hoyler 1907 Autocatalytic action of $H^{\cdot}$. ZPC l 404.
Hudson 1908 Invertase action. JACS xxx 1160, 1564.
1910 PH and invertase. JACS xxxii 1220.
Hüfner 1903 Dissociation of oxyhemoglobin. AAP 217.
—— & Gansser 1907 Osmotic pressure of hemoglobin. Mol. wt. = 16321. AAP 209.
Huenekens 1914 Effect of diet on PH of infant's stomach. KZ xi 297.
Hughes, A. L. 1914 "Photo-Electricity." Cambridge U. Press.
Hulett 1901 Surface tension and solubility. ZPkC xxxvii 385.
1903 Osmotic pressure and capillarity, tensile strength of water. ZPkC xlii 353.

Hutchinson 1915 Resistance of Paramecium. JEZ xix 211.
Hyde 1904 Action current of cell division. AJP xi 52.
—— & Spreier 1915 Light and development. AJP.
Hyman 1916 HCN and oxidation. AJP xl 238.
Iscovesco 1910a Catalase cathodic. Pepsin anodic. BZ xxiv 53.
1910b Surface tension of emulsions. CRB lxix 537.
1910c Cholesterin inhibits action of soap and lecithin by combining with them. CRB lxix 566.
Ishizaka & Loewi 1905 Ca antitoxic to muscarin. ZPk xix 593.
Itami & Pratt 1909 Oxidation and cell structure. BZ xviii 303.
Jackson 1915 Growth of organs of rat. JEZ xix 99, AJA xviii 75.
Jacobs 1909 Dessication of rotifers. JEZ vi 207.
Jacoby 1910 Enzymes of egg and sperm. BZ xxvi 336, 536.
—— & Golowinski 1908 Precipitate of caffein in muscle. AEP lix (sup) 286.
Jahnson-Blohm 1913 Enzymes displaced from surface by anaesthetics and saponin; and activity restored by the latter. ZPC lxxxii 178.
Januschke 1913 Formation of exudates inhibited by anaesthetics. WKW (No. 20).
Jennings 1904 Tropisms. CIWP (No. 16).
1906 "The Behavior of the Lower Organisms." Columbia U. Press.
1914 Paramecium swims backward consinuously in stimulating solution. JCNP xiv 442.
Jensen & Fischer 1913 Hydration in muscle. ZAP xiv 320.
Joel 1915 Anaesthetics retard increase in conductivity of erythrocytes. AGP xlxi 5.
Johannsen 1906 Etherization causes lilacs to sprout earlier. "Das Aetherverfahren beim Fruehtreiben." Jena.
Johnston 1916 CO_2 in water. JACS xxxviii 947.
Jones, H. 1897 "Freezing Pt., Boiling Pt. and Conductivity Methods." Easton, Pa.
1907 Hydrates in solution. CIWP (No. 60).
Jones, W. 1913 Surface tension of solids. ZPkC lxxxii 448.
Jordan 1913 "Vergleichengle Physiologie Wirbelloser Tiere." Jena.
Jordis 1908 Colloids are electrolytes. KZ ii 361, iii 13.
Jorissen & Woudstra 1911 Beta rays coagulate positive sols. KZ viii 8.
Joseph & Meltzer 1911 Abolition of irritability with Ca and restoration with Na. AJP xxix 1.
—— & Austin 1900 Toxicity and ionization. JPC iv 76.
—— & True 1891 Toxicity of Cu ions. BG xxii 81.
1896 n/6400 $H^{\cdot}$ toxic. JAMA xxvii 138.
Kanda 1914 Reversibility of geotropism. AJP xxxv 162.
1916 Geotropism. BB xxx 57.
Kanitz 1903 $H^{\cdot}$ and invertase. AGP c 547.
1906 Dissociation of amino acids. ZPC xlvii.
1907 Dissociation of tyrosine and phenylalamine. AGP cxviii 539.
1915 Temp. Coef. of respiration. IZPB ii 272.
Kastle 1910 Oxidases. BHL (No. 59).
—— & Loevenhart 1900 Synthesis of fat by pancreas lipase. ACJ xxiv 491.
1903 HCN may hasten catalysis. ACJ xxix 397.
1907 Synthesis of fat by pancreas lipase. JBC ii 427.
Katy 1896 Salts in frog's muscle. AGP lxiii 1.
Katzenellenbogen 1906 Permeability of the gut. AGP cxiv 522.
Kaufler 1903 Surface tension and osmotic pressure. ZPkC xliii 686.
Keith, A. & Flack 1907 Conduction in heart. JAP xli 172.
Keith, N., Rowntree & Garaghty 1915 Blood volume. AIM xvi 547.
Kidd 1916 CO_2 inhibits cell respiration. PRS (B) lxxxix 136.

Kirsch 1912 Surface tension of solutions increasing permeability of yeast to invertase = .5. BZ xl 152.

1912 Surface tension of cells. BZ xl 152.

Kite 1913 Permeability of protoplasm. BB xxv 1.

Knoblach 1901 Charge of colloids. ZPkC xxxix 225.

Knüpffer & Bredig 1898 Temperature and direction of reaction. ZPkC xxvi 255.

Knowlton 1911 Addition of gelatine to blood decrease secretion of urine. Starch solution not effective. JP xliii 219.

Koch & McLean 1910 Anaesthetics and size of lipoid particles. JPET ii 249.

Kodis 1898 Undercooling of muscle. ZP xii 593.

1901 Conductivity of dying muscle. AJP v 267.

Köhler 1904 Ultra violet microscopy. ZWM xxi 129, 273.

Koelichen 1900 Polymerization in concentrated solution only. ZPkC xxxiii 129.

1895 Hematocrit and osmotic pressure. AAP 154.

Koeppe 1897 Erythrocytes increase ionization of CO_2 and HCO_3' exchanged for Cl' of serum. AGP lxvii 189.

1900 Physikalische Chemie in der Medizin. Wien.

1905 Erythrocytes in hematocrit appear laked. AGP cvii 183, 187.

Kohlrausch 1907 Speed of ions. ZEC xiii 333.

—— & Heydweiller 1894 Conductivity of $H_2O = .0000385$ at 18°. ZPkC xiv 317; APk liii 209.

Koltzoff 1908 Swelling of spermatozoa. AZ ii 1.

1912 Effect of salts on contractile stalk of Zoothamnium. AGP cxlix 327.

1914 Optimum PH for phagocytosis. JZPB i 82.

Koopman 1915 Enzymes. JZPB ii 266.

Konikopf 1913 PH of blood. BZ li 200.

Koranyi, Von 1897 Δ of blood varies with CO_2. ZKM xxxiii 1.

Kovacs 1902 Δ of blood varies with CO_2. BKW xxxix 362.

Kozawa 1914a Hemolysis greatest at isoelectric point. Reversal of cataphoresis. PH of $H_2PO_4 + NAH_2PO_4$. BZ lx 146.

1914b Permeability of erythrocytes to sugar. BZ lx 231.

Krafft 1896 Soaps are colloids. BDCG xxix 1328.

Kreibich 1910-1 Hg does not markedly affect PH of blood. WKW xxiii 355 xxiv.

Krogh 1904 CO_2 tension of sea water. Meddelelser om Grönland, xxvi 333. Copenhagen.

1908 Micro-tonometer. SAP xx 259, 279.

1910 Absorption of O_2 and CO_2 by water. SAP xxiii 224.

1910 Blood gas exchange in lungs. SAP xxiii 248.

1914 Temperature coefficient of development. ZAP xvi 163, 178.

—— & Krogh 1910 CO_2 and O_2 tension of arterial blood. SAP xxiii 179.

Kuehne 1864 Violet cells of Tradescantion become red at anode and green at cathode (due to metal electrode). "Untersuchungen ueber das protoplasma und die Contractilitaet." Leipsic.

Küster, E. 1910 Formation of plasma membrane. ZBt ii 689; AE xxx 351.

1911 Plant cells absorb many sulfonic acid dyes. JWB l 261.

1913 Liesegang's rings. KZ xiii 192.

Küster, F. 1894-5 Adsorption formula. ZPkC xiii 445; AC cclxxxiii 360.

Kyes 1902 Cobra hemolysin activated by lecithin. BKW 886.

Lachs & Michaelis 1911 Adsorption of NaCl not antagonized by non-electrolytes. KZ ix 275; ZEC xvii 1.

Landolt-Boernstein-Roth 1912 Physikochemische Tabellen. 4th Ed. Berlin.

Landsteiner 1913 Blood shadows precipitate at isoelectric point. BZ l 176.

—— & Jagec 1904 Precipitation of emulsoids with suspensoids; adsorption compounds of immune bodies. MMW (No. 27).

—— & Uhlirz 1905 Adsorption of colloids irreversible. ZB xl 265.

Lapique 1909 Nernst's theory of stimulation. CRA cxlix 871; JPP xi 1009, 1035.

—— & Legendre 1913 Diameter of neuraxon and conduction. CRA clvii 1163.

Laquer & Sackur 1903 Viscosity of casein increased by acids and alkalis. BCPP iii 193.

Leathes 1895 Δ of lymph greater than blood. Infusion of hypertonic NaCl increases blood volume. JP xix 1, 418.

LeBlanc 1913 No Tyndall phenomenon of sugar solutions. ZEC xix 794.

Lee 1903 Alcohol increases rhythm of medusa. AJP viii (p. xix).

—— & Salant 1902 Alcohol and muscle. AJP viii 61.

Lehmann 1884 Mice and frogs die in compressed oxygen. Oxidation of pyrogallol independent of O_2 pressure. AGP xxxiii.

Lepeschkin 1908 Light increases permeability of plant cells. BDBG xxvi (a) 198, 231, 724.

  1910 Plasma membrane and Traube's membrane are sols. BDBG xxviii 383.

  1911 Composition of plasma membrane. BDBG xxix 247.

Lesser 1907 Effect of temperature on skin current. AGP cxvi 124.

Levy, Rowntree & Marriott 1915 Indication method for blood. AIM xvi 3.

Levy & Rowntree 1916 Buffer value of blood. AIM xvii 525.

Lewis, G. 1906 Color change of Co or Cu solutions due to hydration of ions. ZPkC lvi 223.

  1908 $P = 12.06\,\Delta - .021\,\Delta^2$ JACS xxx 668.

—— & Keyes 1912 Sol. Ten. of K and Li. JACS xxxiv 122.

—— & Lacey 1914 E_H Cu $= -.3347$. JACS xxxvi 804.

—— & Randall 1914 Free energy of H. JACS xxxvi 1969.

—— & Rupert 1911 Solution tension of Cl. JACS xxxiii 299.

Lewis, M. 1916 Sea water as culture medium. AR x 287.

Lewis, W. 1908 Haptogen membranes. PM (6) xv 499.

  1909 Surface tension between oil and water. PM (6) xvii 466.

    Electric charge of oil drops in water. KZ iv 211.

  1910 Surface tension between liquids, adsorption of NaCl. ZPkC lxxiii 129.

Lewites 1907 Swelling of gels. KZ ii 166.

Liebreich 1890 Surface films and chemical reaction. ZPkC v 529.

Liesegang, R. 1911 Liesegang's rings. AE xxxiii 328.

L'Hermite 1855 Permeability by solution in membrane. ACP (3) xliii 420.

Lillie, F. 1913 Acids cause aggregation and alkalis agglutination of sperm. JEZ xiv 515.

Lillie, R. S. 1902 Oxidation at nucleus. AJP vii 412.

  1903 Spermatozoa are electronegative. AJP viii 273.

  1905 Model of mitosis. AJP xv 46.

  1907 Osmotic pressure of proteins reduced by salts and increased by $H^{\cdot}$ and OH'. AJP xx 127.

  1908 Heat-Parthenogenesis. JEZ v 375.

  1909a $H^{\cdot}$ produces polarization of plasma membrane. Lyotrope series and cytolysis, $MgCl_2$ decreases permeability. AJP xxiv 14, 36.

  1909b Artificial parthenogenesis and permeability. BB xvii 188.

  1909c Effect of salts on cilia. AJP xxiv 459.

  1910 Lyotrope series, cytolysis and parthenogenesis. AJP xxvi 106, 126.

  1911a Permeability, stimulation and contraction. AJP xxviii 197.

  1911b Fertilization. BB xxii 328.

1912a Anaesthetics may increase size of lipoid particles in plasma membrane by dissolving in them. AJP xxx 372.
1912b Anaesthetics decrease toxicity of pure NaCl. AJP xxx 1.
1913a Anaesthetics inhibit increase in permeability. AJP xxxi 255.
1913b Oxidation in blood cells increased by induction shocks. JBC xv 237.
1913c Cell division. JEP xv 23.
1914a Rate of conduction. AJP xxxiv 414.
1914b Anaesthetics vs. salts. JEZ xix 591.
1914c Cell narcosis. JBC xvii 121.
1914d Stimulation and permeability. PSM 579.
1916a Fertilization increases permeability of egg to water. AJP xl 249.
1916b Rate of nerve impulse varies as electric conductivity of medium. AJP xli 126.
1916c Anaesthesia. BB xxx 311.
Linder & Picton 1895 Antagonism of cations in precipitation of sols. Electric charge, dispersion and freezing out of colloids. JCS lxvii 63, 73.
1897 $Al_2(OH)_6$ and $Fe_2(OH)_6$ sols are electro-positive. Conductivity of suspensoids same as water. Mutual precipitation of sols of opposite sign. JCS lxxi 568, 572.
1905 Ultramicroscopy of coagulation. JCS lxxxvii 1911.
Lipscomb & Hulett 1916 Electrolytic calomel. JACS xxxviii 20.
Locke 1900-1 Locke's solution. ZP xiv 670, xv 490.
Lodholz 1913 All or none law. ZAP xv 269.
Loeb, J. 1889 Heliotropismus der Tiere. Würzburg.
1897 Stimulating current must run longitudinally in nerve. AGP lxvii 483, lxix 99.
1898a Swelling of muscle. AGP lxix 1.
1898b OH' necessary for egg development. AE vii 631.
1900 Artificial parthenogenesis. AJP iii 135.
1902 Toxicity of $Na^{\cdot}$ and antitoxin action of bivalent ions. AJP vi 411.
1903 Toxicity of sugar. AGP xcvii 394.
1906 $Ca^{\cdot\cdot}$, $Sr^{\cdot\cdot}$ and $Ba^{\cdot\cdot}$ cause rhythmic contraction of center medusa. JBC i 427.
1907 Antitoxic action of $Ca^{\cdot\cdot}$ on $Na^{\cdot}$ in cytolysis of sea urchin eggs. BZ v 351.
1911a Fundus eggs live in distilled water. AE xxxi 654.
1911b Antitoxic action of $Ca^{\cdot\cdot}$ and $Sr^{\cdot\cdot}$ on $K^{\cdot}$ and $Na^{\cdot}$ and of $Na^{\cdot}$ on $K^{\cdot}$. BZ xxxi 450; xxxii 155, 308.
1912a Toxicity of sugar. JBC xi 415.
1912b Increased permeability to water. BZ xlvii 127.
1913 Artificial Parthenogenesis and Fertilization. Chicago.
1914a "Fertilizin" not necessary for fertilization. JEZ xvii 123.
1914b Bunsen-Roscoe law and phototropism. ZP xxvii 80.
1915a Salts antitoxic to acids. JBC xxiii 139.
1915b Ca and permeability. JBC xxiii 423.
1916 Phototropism. JEZ xx 217.
—— & Budgett 1897 Galvanotropism. AGP lxv 518.
—— & Beutner 1912 Current of injury in plants. BZ xli 1, xliv 303.
1913 Current of injury. BZ li 288.
1914 Bioelectric currents. BZ lix 201.
—— & Cattell 1915 Antagonism of salts. JBC xxiii 41.
—— & Ewald 1916 Chemical stimulation of nerve. JBC xxv 377.
—— & Gies 1902 Toxic and antitoxic action of ions. AGP xciii 246.
—— & Wasteneys 1910 Oxidation in egg. Medusae anaesthetized by Mg use more O_2. BZ xxviii 340, 350.

1911a Bases and alkaline salts increase oxidation. BZ xxxviii 410.

1911b Bromide intoxication of fish. BZ xxxix 185.

1912a Adaption to heat. JEZ xii 543.

1912b Heart rhythm reduced by reduction of O_2. BZ xl 277.

1913 Anesthetics reduce cell division more than oxidation. JBC xiv 517.

1915a Effect of poisons on Δ of Fundulus. JBC xxi 223.

1915b Osmotic pressure of cell. JBC xxiii 157.

1915c Phototropism. JEZ xix 23.

Loeb, L. 1901 Phagocytosis. AGP cxxxi 465.

Loeb, W. & Higuchi 1910 PH of serum of placenta. BZ xxiv 92.

Loew 1889 Synthesis of sugar from formaldehyde. BDCG xxii 470.

1892 Antagonism of Ca and Mg. F 368.

Loewe, F. 1912 Water interferometer. KZ xi 226.

Loewe, S. 1912 Methylene blue adsorbed by lecithin (in chloroform). BZ xlii 150-218.

1913 Anesthetics reduce conductivity of artificial lipoid membranes. BZ lvii 161.

Loewy 1894 Alkali albuminate more concentrated in corpuscle than in serum. AGP lviii 462.

—— & Zuntz 1894 CO_2 increases diffusible alkali in serum. AGP lviii 511.

Loomis 1894 Δ of NaCl. APk li 515.

1897 Δ of NaCl. APk ix 527.

—— & Acree 1911 Calomel electrode. ACJ xlvi 585.

Lorant 1914 Surf.-Ten. between Liquids. AGP clvii 211.

Lorenz & Boehi 1909 Dissociation of water. ZPkC lxvi 749.

Lottermoser 1907 Adsorption by filters. ZPkC lx 458.

1908 Freezing out of colloids. BDCG xli 3976.

1910 Peptization and reversal of charge of sols. KZ vi 78.

—— & Rothe 1908 Impurities of sols necessary for stability. ZPkC lxii 359.

Löwenherz 1896 Influence of alcohol on dissociation of water. ZPkC xx 283.

Lubs & Clark 1915 New indicators. JWAS v 609.

Lucas 1909a Temperature coefficient of nerve conduction. JP xxxvii 112.

1909b Rise of neg. var. prop. to rate of transmission. JP xxxix 207.

1909c All or none law. JP xxxviii 113.

Ludwig 1849 Swelling of colloids. ZRM viii.

Ludloff 1895 Galvanotropism due to reversal of cilia at cathode. AGP lix 523.

Lunden 1908 Ampholytes. JBC iv 267.

Lundsgaard 1912 PH of blood. BZ xli 247.

Lusk 1912 "The Elements of the Science of Nutrition." 3rd Ed. Phila.

1915 Influence of food on metabolism. JBC xx 555 (and p. viii).

Luther 1896 Adsorption potential of colloids. ZPC xix 529.

—— & Forbes 1909 Photochemistry. JACS xxxi 770.

Lyon 1898 Otocyst. JCNP viii 238.

1901 Rhythmic production of CO_2 and sensibility to heat and cold (eggs). AJP xi 52.

1902 Cell division and rhythmical susceptibility to KCN. AJP vii 56.

1903 Artificial parthenogenesis. AJP ix 308.

1904 Rheotropism. AJP xii 147.

1905a Geotropism. AJP xiv 421.

1905b Density of eggs. AJP xxiv 244.

1906 Geotropism. BB xii 21.

1909a Rheotropism. AJP xxiv 244.

1909b Catalase and fertilization. AJP xxv 199.

—— & Shackell 1910a Autolysis and fertilization. JBC vii 371.
1910b Fertilization and permeability. S xxxii 249.
Macallum 1904 Blood salts same as Cambrian sea. Trans. Can. Ins.
1905 Microanalysis of K. JP xxxii 95.
1908 Microanalysis. EP vii 552.
1911 Surface tension. EP xi 595.
1912 Surface Tension and Vital Phenomena. Toronto Univ. Press (Physiol.) No. 8.
1913 Muscular energy. JBC xiv (p. ix).
—— & Benson 1909 Δ of urine may be as low as .07. JBC vi 87.
McCallum 1905 Regeneration in plants. BG.
McCaskey 1908 Viscosity of blood. JAMA li 1653.
McClendon 1907 Removal of chromatin from eggs with "mechanical hand." BB xii 141.
1908 Chromatin unnecessary for cell division. AE xxvi 662.
1909a Conduction of excitation in ameba. "Mechanical hand." JEZ vi 85 (Plate I).
1909b Nucleus is such as may act as statocyst in geotropism of paramecium. JEZ vi 87 (Plate II).
1909c Resistance of cell structure to centrifugal force and separation of phases of different density. AE xxvii 254 (Plate VI).
1909d Physical differences between cells. AJP xxiii 460.
1909e The association of lecithin with protein in the frog's egg. AJP xxv 195.
1909f Artificial parthenogenesis produced in a variety of ways. S xxx 454.
1910a Frog's egg composed of 4 or more phases. AZ v 385.
1910b Protoplasm is anodic and mitotic figure comparatively rigid. AE xxxi 80.
1910c Fertilization increases the conductivity of the egg. S xxxii 122.
1910d Fertilization decreases the sensitiveness of the egg to electrolysis and increases its sensitiveness to hypertonic sugar solution. S xxxii 317.
1910e Fertilization or artificial stimuli increases the permeability of the egg. AJP xxvi 240.
1911a Fertilization membrane. S xxxiii 387.
1911b Electric shock causes frog's egg to segment. S xxxiii 629.
1911c Ameboid motion and permeability. AGP cxl 271.
1912a Pure NaCl solution increases permeability of Fundulus eggs. Egg membrane or shell is freely permeable. AJP xxix 289.
1912b Artificial parthenogenesis of frogs and toads. AJP xxix 298.
1912c Increased conductivity of tetanized muscles. AJP xxix 302.
1912d Osmosis and surface tension in physiology. BB xxii 113.
1912e Alkaloids increase permeability of eggs. AJP xxxi 131.
1912f Echinochrome is not a respiratory pigment. JBC xi 435.
1913a Increase in permeability of Fundulus eggs to salts. S xxxviii 280.
1913b Surface tension and cell division. AE xxxvii 233.
1914a Increase in permeability of frog's egg. S xl 70.
1914b Antagonism of salts and anesthetics. S xl 214.
1914c Permeability-increase of Fundulus eggs to Cl', $\mathrm{Na}^{\cdot}$, $\mathrm{K}^{\cdot}$, $\mathrm{Ca}^{\cdot\cdot}$ and $\mathrm{Mg}^{\cdot\cdot}$. IZPB i 28.
1914d Electric charge of protoplasm. IZPB i 159.
1914e Fertilization membrane. IZPB i 163.
1914f Osmotic swelling of frogs. IZPB i 169.
1914g Permeability of cells. CIWP (no. 183) 123.
1915a Fertilization or electric stimulus increases permeability of frog's egg to Na, K, Mg, Ca, Cl and SO_4. AJP xxxviii 163.

1915b Pure NaCl solution increases permeability of fish eggs to salts, and anesthetics inhibit this change. AJP xxxviii 173.
1915c Hydrogen electrodes. AJP xxxviii 180.
1915d Potentiometer for hydrogen electrode. AJP xxxviii 186.
1915e PH of stomach and duodenum of adult and infant. AJP xxxviii 191.
1915f Peptic digestion occurs in infant's duodenum. JAMA lxv 12.
1915g Pure oxyhemoglobin oxidizes a-napthol, aloin or p-phenylene diamine. JBC xxi 275.
1916a Hydrogen electrode and tonometer and conversion table. JBC xxiv 519.
1916b Colored indicator chart. MRR xxii 333.
—— & Magoon 1916 Combined tonometer and hydrogen electrode. JBC xxv 669.
—— & Mitchell 1912 Parth. and incr. oxidation of egg caused by OH′. JBC x 459.
McClintic 1912 Phenol coefficient of disinfectants. BHL (No. 82).
McCoy 1903 Dissociation of CO_2. ACJ xxix 437.
MacDonald 1905 Microanalysis of chlorides. PRS (B) lxxvi 272, 322.
McDougall 1898 Theory of muscle contractions. JAP xxxi, xxxii 193.
1908 Muscle contraction. JAP xxxii 193.
McGuigan 1904 Solution tension and enzyme action. AJP x 444.
MacInnes 1914 Transference no. and diffusion potential. JACS xxxvi 878, 2301.
McIntosh 1897 Lyotrope series. JPC i 473.
Mackenzie 1910 Gravitation. Bull. Minnesota Acad. Sc. iv 385.
McPhedran 1913 Hemolysis. JEM xviii 527.
Magnus 1900 Capillaries permeable to proteins. AEP xliv 68.
1901 Blood volume restored after increase by transfusion. AEP xlv 210.
——, Sorgdrager & Leeuwen 1914 Lungs impermeable to NH_3. AGP clv 275.
Manape & Matula 1913 Protein binds little Cl′ of NaCl. BZ lii 369.
Mansfield 1908 Obesity and rate of resorption and circulation in narcosis. AIPd xvii 347.
1909 Narcosis and O_2 solubility. AGP cxxix 69.
Marc 1911-3 Adsorption-saturation. ZPkC lxxvi 58; lxxxi 641.
1913 Adsorption and solid solution. ZPkC lxxxi 641.
Marriott 1916 CO_2 tension with indicator. JAMA lxvi 1594.
Masel 1913 Acidosis in diabetic coma. ZKM lxxix 1.
Masing 1912 Permeability of erythrocytes to sugar. AGP cxlix 227.
1914 Temperature coefficient of diffusion of sugar into erythrocytes. AGP clvi 401.
Massart 1889 Turgor regulation. AB ix 515.
Mast 1911 "Light and the Behavior of Organisms." N. Y. Wiley.
1914 Tropisms. BC xxxiv 641.
—— & Root 1916 Surface tension and phagocytosis. PNAS ii 188; JEZ xxi 1.
Mathews, A. 1902a Nerves lose excitability in non-electrolytes and regain it in salt solutions. S xv 492.
1902b Current of injury. AJP viii 294.
1904a Solution tension and toxicity. Fundulus egg impermeable to NaCl. AJP x 290.
1904b Oxidising anions act best in acid and cations in alkali. AJP xi 237.
1904c Osmotic stimulation and solution tension. Ba stimulates nerve. AJP xi 455.
1905a Electrolytic solution tension and toxicity. AJP xii 419.

1905b Solution tension and precipitation of sols. AJP xiv 203.
1907a Toxicity of NH_4 salts due to hydrolysis. AJP xviii 58.
1907b Cell division. AJP xviii 89.
1909 Oxidation of sugar. JBC vi 3.
1914 Narcosis and residual valence. IZPB i 433.
1915 Physiological Chemistry. N. Y. Wood.
—— & Longfellow 1910 Chemical theory of permeability. JPET ii 201.
Mathews, S. & Brooks 1910 Action of $MgSO_4$. JPET ii 88.
Matthews, D. 1916 P in sea water. JMBA xi 122.
Mathison 1911 $H^{\cdot}$ stimulates vasomotor center. JP xlii 283; xli 416.
1911 Effect of PH on reduction of arterial blood. JP xliii 5.
Maxwell 1905 Effect of salts on cilia. AJP xiii 154.
Mayenburg 1901 Turgor regulation. JWB xxxvi 381.
Mayer, A. G. 1906 Pulsations of medusa (Cassiopea). CIWP (no. 47).
1914a Electrolytes and rate of nerve conduction. CIWP (No. 183) 25.
1914b Effect of temperature on animals. CIWP (No. 183) 1.
1915 Nerve conduction. PNA i 270.
1916 Nerve conduction. AJP xxxix 375.
Mayer, A. d. 1902 "Gärungschemie."
Mayerhofer & Pribram 1909 Diffusion of colloids. ZEP vii.
—— & Stein 1910 Sugar increases permeability of gut. BZ xxvii 376.
Means, Newburgh & Porter 1915 Increased Alv. CO_2 in infections. clxxiii 742.
Meek & Eyster 1912 Positive variation of auricle after vagus stimulation. AJP xxx 271.
Meigs 1908a Theory of muscle contraction. AJP xxii 477.
1908b Structure of muscle. ZAP viii 81.
1909a Heat rigor of muscle. AJP xxiv 1, 178.
1909b Water rigor. JP xxxix 384.
1910 Swelling, and contraction of muscle. AJP xxvi 191.
1912a Smooth muscle. QJEP v 55.
1912b Structure of smooth muscle. AJP xxix 317.
1912c Smooth muscle not semipermeable. JEZ xiii 497.
1912d Muscle permeable to KCl. JEZ xiii 520.
1914a Clam adductor. JBC xvii 81.
1914b Swelling of muscle. AGP clviii 1.
1915a Phosphate membranes. AJP xxxviii 456.
1915b Ash of clam muscle. JBC xxii 493.
—— & Atwood 1916 KCl selectively absorbed by muscle. AJP xl 30.
—— & Ryan 1912 Ash of smooth muscle. JBC xi 401.
Mellor 1909 "Higher Mathematics for Students of Chemistry, etc." 3rd Ed. Longmans.
Meltzer & Auer 1905 Anesthesia with Mg. AJP xiv 361.
1908 Ca vs. Mg. ZP xxi 788.
Mendel, J. Gregor 1865 Mendel's Law. VNVB iv.
Mendel, L. & Thatcher 1903 Secretion increases lymph of pancreas. AJP ix (p. xv).
Mendelssohn 1916 Leucocytes are pos. galvanotropic. CRA lxii 52.
Menten 1915 PH of stomach. JBC xxii 391.
—— & Crile 1915 PH of blood. AJP xxxviii 225.
Menz 1908 Submicrons in gelatine. ZPC lxvi 129.
Merrill 1915 Exosnose from roots. AMBG ii 507.
Metcalf 1905 Haptogen membranes. ZPC lii 1.
Meyer, H. 1899 Theory of narcosis. AEP xlii 109.
1901 Narcosis and partition coefficient. AEP xlvi 338.
1909 Anesthetics increase permeability and cause mixing inside cells. MMW lvi 1577.
Meyer, K. 1911 Bacterial protease. BZ xxxii 274.

Meyer, L. & Cohn 1911 Water retention favored by Na and inhibited by Ca. ZK ii 360.
Meyerhof 1911a Oxidation of fertilized egg greater in pure NaCl. BZ xxxiii 291.
1911b Thermodynamics of eggs. BZ xxxv 159-184.
1912a Thermodynamics of cells. AGP cxlvi 159.
1912b Reduction of dyes by dead cells. AGP cxlix 250.
1914a Urethane retards oxidation of fertilized more than unfertilized eggs. Anesthetics do not lower osmotic pressure of proteins. AGP clvii 251.
1914b H_2O_2 catalysis by Pt. inhibited by anesthetics. AGP clvii 307.
1915 Anesthetics and enzymes. JZPB ii 394.
Michaelis 1908 Adsorption of colloids irreversible. BZ xii 26.
1908 Solution tension and polarization. ZEC xiv 353.
1909 Calculation of isoelectric point. BZ xix 181.
1912 Isoelectric point of proteins. Nernst-Festschrift. 308.
1914 "Die Wasserstoff ionenkonzentration." Berlin.
—— & Davidsohn 1910 Indicators for stomach. ZEP viii 398.
1910 Isoelectric point. BZ xxx 143.
1911 Optimum PH for invertase. BZ xxxv 386.
1913 Gelatine stains with acid dyes in acid solution. BZ liv 323.
—— & Ehrenreich 1908 Acid dyes and invertase adsorbed by clay but not by kaolin. BZ x 283.
—— & Menten 1913 Action of invertase inhibited by fructose. BZ xlix 333.
—— & Mostynski 1910 Viscosity and ionization of proteins. BZ xxv 401.
—— & Perkins 1914 "Dynamics of Surfaces." London.
—— & Pincussohn 1906 Ultramicroscopy of colloid reactions. BZ ii 251.
—— & Rona 1907 Protein precipitated by dialysed iron. BZ ii, iii, iv, v.
1908a Osmotic compensation. BZ xiv 476.
1908b Adsorption of colloids irreversible. Competitive adsorption. BZ xv 196.
1909a Competitive adsorption. BZ xvi 489.
1909b Effect of salts on Congo red. BZ xxiii 61.
1910 Isoelectric point of denatured albumin. BZ xxvii 38.
1912 Isoelectric points of emulsions. BZ xxxix 481.
—— & Takehashi 1910 Hemolysis and isoelectric point. BZ xxix 439.
Miculicich 1910 Effect of ions on hemolysis by anesthetics. ZP xxiv 523.
Millikan 1910 Charge of an ion. PM xix 209.
1916 Equation for contact electricity. PR vii 18.
Milroy 1914 PH and respiratory center. QJEP viii 141.
Mines 1911a Ca, Mg, Sr and Ba inhibit the stimulating action of K on muscle. JP xlii 251.
1911b Ppt. of As_2S_3 by cations. Action of rare earth salts on emulsoids and reversal of emf of concentration cell with gelatine diaphragm. JP xlii 309.
1911c PH of pecten blood = 6.7. Heart stops in diastole in acid, systole in alkali. JMBA ix 171.
1912 PH for heart of pecten. JP xliii 467.
1913a Action of ions. JP xlvi 188.
1913b Dynamic equilibrium of the heart. JP xlvi 349.
Milner 1907 Adsorption and surface tension. PM (6) xiii 96.
Mitscherlich 1893 Ignition point of H_2+O_2 raised by pressure. BDCG xxvi 399.
Moldovan & Weinfurter 1904 Anesthetics reduce cell respiration. AGP clvii 571.
Momose 1915 Alv. CO_2 and PH of blood. BJ ix 485.

Montgomery 1878 St. Thos. Hos. Repts. London, n. s. ix.
1881 Muscle contraction and ameboid motion. AGP xxv 497.
Mond, Ramsay & Shields 1898 Absorption of H_2 by metals. PRS lxii 290.
Moore, A. 1910a Temp. coef. of cytolysis and hemolysis = 200 for 10°. QJEP iii 257.
1910b Temp. coef. of regeneration. AE xxix 145.
Moore, B. 1914 Iron and photosynthesis. PRS (B) lxxxvii 556.
—— & Roaf 1906 Lipoids and proteins precipitated by anesthetics in low concentration. PRS (B) lxxvii 86.
1907 Osmotic pressure of colloids. BJ ii 34.
1908 More salts dialyze from serum than from erythrocytes. BJ iii 55.
—— & Webster 1913 Photosynthesis of formaldehyde from CO_2 and H_2O. PRS (B) lxxxvii 163.
—— & Whitley 1909 Oxidases. BJ iv 136.
Morawitz, H. 1910 Adsorption of $HgCl_2$ by erythrocytes. KZ vi 259.
1910 Adsorption of antiseptics by bacteria. Reduction of heavy metals by adsorption. KCB i 301.
Morawitz, P. 1909 Oxidation in blood. AEP lx 298.
Morgulis & Fuller CO_2 in sea cannot be titrated with phenolphthalein. JBC xxiv 31.
Morgan, J. 1915 Measurement of surface tension. ZPkC.
Morgan, T. & Stockard 1907 Effects of salts and sugar on frog's egg. BB xiii 272.
Morse, H. 1914 Osmotic pressure of sugar solutions. CIWP (No. 198).
—— & Horn 1901 Osmotic membranes. ACJ xxvi 80.
Morse, M. 1912a Eggs in sperm extracts. JEZ xiii 471.
1912b Parthenogenesis. JEZ xiv 471.
1915 Halogens and enzymes. JBC xxii 125.
1916 Autolysis is autocatalyzed by $H^{\cdot}$. JBC xxiv 163.
Moruzzi 1910 Urea liberates NH_3. BZ xxviii 97.
Mourson & Schlagdenhauffen 1882 Sea urchin blood. CRA xcv 791.
Mouton 1897 Turgor regulation. CRA cxxv 407.
Müller, C. 1912 Absorption of gases by non-electrolyte solutions. ZPkC lxxxi 483.
Müller-Thurgau 1880 Capillarity and undercooling. LJ ix 176.
1886 Undercooling of plants. LJ xv 490.
Murlin, Edelmann & Kramer 1913 CO_2 and O_2 of blood in stasis. JBC xvi 79.
—— & Kramer 1913 Respiration in glycosuria. JBC xv 365.
Myers & Acree 1913 H_2 electrode. ACJ l 396.
Nägeli 1855 "Pflanzenphysiologische Untersuchungen."
1885 "Pflanzenphysiologische Studien." Zurich.
Nagel 1909 "Handbuch der Physiologie des Menschen." Braunschweig. F. Vieweg.
Nasse 1869 Swelling of muscle. AGP ii 97.
Nathansohn 1904 Lecithin changes permeability when wet. JWB xxxix 607.
Nemec 1900 Geotropism of plants. BDBG xviii 241.
Neilson 1906 Platinum black poisoned by HCN in splitting of amygdalin. AJP xv 148.
Nernst 1888 Formula for electrode and diffusion potential. ZPkC ii 613, 639.
1889 Formula for diffusion potential. ZPkC iv 129.
1890 Permeability and solution in plasma membrane. ZPkC vi 37.
1892 Solution tension and polarization. ZPkC ix 137.
1897 Tesla currents do not stimulate. APk lx 600.
1911 "Theoretical Chemistry." Trans. of 6th Ed. Macmillan.
—— & Barratt 1904 Verification of Nernst's theory of stimulation. ZEC 663.

—— & Riesenfeld 1902 Diphasic concentration cells. APk (4) viii 600.
Neuberg 1911 "Die Harn sowie die uebrigen Körperflussigkeiten." Berlin.
Neufeld & Haendel 1908 Hemolysins are cytolytic. Bile salts dissolve blood shadows. AKG xxviii 572.
Nicloux 1913-14 Absorption of CO_2 by blood. CRA clvii 1425, clviii 363.
Nishi 1910 Sugar is secreted by glomeruli and resorbed by tubules. AEP lxii 329.
Noguchi 1902 Combination of lecithin and saponine. UPMB (Nov.).
  1905 Snake venom protects erythrocytes. JEM vii 191.
Nolf 1904 Δ of venous and arterial blood. AB xx 1.
Norton & Hsu 1916 Disinfection. JID xviii 180.
Nothmann-Zucherkandl 1912 Protoplasmic streaming quickly stopped with anesthetics but continues weeks without O_2. BZ xlv 412.
  1915 Toxicity of anesthetics increased by salts. JZPB ii 19.
Novak, Leimdoerfer & Porges 1902 Alveolar CO_2 during pregnancy. ZKM lxxv 301.
Noyes 1907 Electric conductivity. CIWP (No. 63).
—— & Blanchard 1900 Color of Co ions and molecules. JACS xxii 726.
——, Melcher, Cooper & Eastman 1910 Conductivity at high temperatures. ZPkC lxx 335.
Okada 1915 Liver bile PH = 7.75, gall more acid. JP I 114.
Oker-Blom 1900 Conductivity of sand + 40% solution = 25% of conductivity of solution. AGP lxxix 510.
  1901 Bioelectric currents. AGP lxxxiv 191.
Onaka 1911 Oxidation of blood platelets. ZPC lxxi 193.
Oppenheimer 1909 Handbuch der Biochemie des Menschen und der Tiere.
  1913 "Die Fermente." Leipzig.
Osborne 1906 Intracellular colloid salts. JP xxxiv 127.
Osterhout 1910 Permeability of cells to Ca. ZPkC lxx 408.
  1911 Permeability of cells. S xxxiv 187.
  1912a Permeability. S xxxv 112.
  1912b Plants requiring Na. BG liv 532.
  1912c Change in permeability. S xxxvi 350.
  1913a Pseudo plasmolysis. BG lv 446.
  1913b Anesthetics and permeability. S xxxvii 111.
  1914a Alkali and permeability. JBC xix 335.
  1914b Acid and permeability. JBC xix 493.
  1915a Acids vs. salts. JBC xix 517.
  1915b Polyvalent ions and permeability. BG lix 464.
  1915c Permeability. AJB ii 93.
  1915d Decrease in perm. BG lix 317.
  1915e Abnormal permeability without death. BG lix 242.
Ostwald, Wm. 1889 Dilution law. ZPkC iii 170.
  1890 Elec. properties of semipermeable membranes. Becquerel phenomenon. ZPkC vi 71, 74.
  1892 Color of ions. ZPkC ix 579.
  1897 Metastable state. ZPkC xxii 302.
  1899 "Grundriss d. allg. Chemie." 3rd Ed. Leipsic.
  1900 Small crystals more soluble than large. ZPkC xxxiv 495.
—— & Luther 1910 "Handbuch Physiko-Chemischer Messungen." 3rd Ed. Leipsic.
Ostwald, Wo. 1905 Gammarus pulex will live in fresh or sea water. AGP cvi 568.
  1907a Disperse systems. KZ i 291.
  1907b Adsorption and toxicity. AGP cxx 19.
  1907c Oxidases sensitive to light. ZB x 130.
  1908 Dissociation of oxyhemaglobin. KZ ii 264.
  1909 Grundriss der Kolloidchemie. Dresden, Steinkopff.

1912 Color of indicators. Dispersion of dyes. KZ x 97.
1913 Viscosity of sols. TFS ix 34.
Otto 1913 Narcosis and evolution. ZB lix 165.
Overton 1895 Plasmolysis and permeability. VNGZ xl 1.
1897 Cells permeable to free alkaloids but not their salts. Permeability to NH_3. ZPV xxii 189.
1899 Permeability of cells. VNGZ xliv 88.
1900 Permeability and partition coefficient. JWB xxxiv 669.
1901 "Studien ueber die Narkose." Jena.
1902a Permeability of muscle 0.65% NaCl for frog. AGP xcii 115.
1902b Na (or Li) necessary for muscle contraction. AGP xcii 346.
1904a Frog's kidneys regulate osmotic pressure. VPMG (NF) xxxvi 277.
1904b Ions restoring excitability of sugar muscle. AGP cv 176.
1907 Frog's egg membrane permeable to crystalloids. II p. 878. Nagel's Handb. d. Physiol. d. Menschen.
Packard 1905 Injection of alkali decreases symptoms of O_2 want. AJP xv 30.
Paine 1912a Permeability of yeast. PRS (B) lxxxiv 289.
1912b Decrease in dispersion of sols and rate of precipitation by electrolysis. KCB iv 24.
Palitzsch 1911a PH by methyl red. BZ xxxvii 131.
1911b PH of sea water. BZ xxxvii 116.
1915 Boric-borax mixtures for PH. BZ lxx 333.
Pantanelli 1904 Osmotic pressure of mould on concentrated KNO_3. Colloid swelling and turgor. JWB xl 303.
1906 Acetates increase permeability of fungi to enzymes. AB v 355.
Pascheles 1898 Coagulation in emulsoid gels. AGP lxxi 333.
Pascucci 1905 Erythrocyte stroma ⅓ lipoid and ⅔ proteins. Artificial lipoid membranes. BCPP vi 543.
1907 Perm. of lipoid mem. to pro-enzymes. BZ vi 358.
Pasteur 1858 Asymmetric fermentation of tartaric acid. CRA xlvi 615.
Paul 1901 Ionization hastens toxic action of heavy metals. ZPkC xxxvii 754.
——, Birstein & Reuss 1910 Adsorption of antiseptics by bacteria. BZ xxix 202.
—— & Krönig 1896 Disinfection with Hg ions. ZPkC xxi 414.
Pauli 1903 Lyotrope series for alkali albumin reversed for acid albumins. Ba, Sr and Ca precipitate proteins irreversibility. BCPP v 27.
1905 Precipitation of emulsoids with heavy metals. BCPP vi 233.
1906 Electric charge of protein. BCPP vii 531.
1907 Heat coagulation of acid albumin. BCPP x 53.
1910 Colloidal state and physiology. AGP cxxxvi 483.
1912 "Kolloidchemie der Muskelkontraktion." Dresden. Steinkopff.
—— & Falek 1912 Caffeine and hydration of proteins. BZ xlvii 269.
—— & Flecher 1912 Precipitation of emulsoids with suspensoids. BZ xli 461.
—— & Handovsky 1908 Ion-protein compounds. BCPP xl 415.
1909 Acid albumin. BZ xviii 340.
1910 Alkali-albumin. BZ xxiv 239.
—— & Matula 1916 Thermo-current of muscle. AGP clxiii 355.
—— & Rona 1902 Swelling of gels. Glycerine and sugar favor and urea inhibits gelation. BCPP ii 1.
—— & Samec 1909 Proteins increase solubility of $Ca_3(PO_4)_2$ and $CaCO_3$ but not of alkali salts appreciably. BZ xvii 235.
1913 Viscosity and optical rotation of emulsoids. KZ xii 222.
——, Samec & Grauss 1914 Optical rotation of protein salts. BZ lix 470.
—— & Wagner 1910 Acids and alkalis increase viscosity of proteins. BZ xxvii 296.

Pavloff 1910 "The Work of the Digestive Glands." Trans. of 2nd Ed. London.
Peabody 1914 No relation of PH to dyspnoea in cardio-renal. AIM xiv 236.
1916 Alv. $CO_2 = 6$ mm in diabetic coma. AJMS cli 184.
Pearl & Gowan 1914 Refractive index of serum. PSEB xii 48.
Pekelharing 1914 Nucleo-protein, Ca and blood coagulation. ZPC lxxxix 22.
—— & Ringer 1911 Cataphoresis of pepsin. ZPC lxxv 282.
Pelet-Jolivet & Anderson 1908 Adsorption and electric charge. KZ ii 225.
Perrin 1904-5 Dielectric constant and contact electricity. Formula for cataphoresis. JCP ii 601, iii 50.
1908 Stokes law and Brownian movement. CRA cxlvi 960, cxlvii 475.
1909 Ultramicroscopy of coagulation. ACP (8) xviii 89.
1911 Avogadro's No. = 6×10^{23} molecules in mol. wt. CRA clii 1165, 1382.
Pfäundler 1905 PH of blood of premature babies = 6.55. Normal = 7.5. AK xli 161, 174.
Pffeffer 1873 "Physiologische Untersuchungen." Leipsic.
1877 "Osmotische Untersuchungen." Leipsic, Engelmann.
—— & Hansteen 1893 Splitting of starch in germinating corn inhibited by sugar. BVGW xlv 421.
Pffeiffer & Von Modelski 1912-3 Amino acids bind NaCl. ZPC lxxxi 329, lxxv 1.
Philip 1914 "Physical Chemistry and Its Bearing on Biology." 2nd Ed. London.
Philips 1907 Effect of solutes on solubility of gases. TFS iii 140.
Phillipson, Hannevart & Thieren 1910 Δ of fresh water animals. AJP ix 460.
Philoche 1908 Maltase action is autocatalytic. JCP vi 213, 355.
Pickering 1910 Haptogen membranes and emulsions. KZ vii 11.
Pictet 1893 Diatoms live at —200°. ASPN xxx 311.
Picton & Linder 1892 Colloids show Tyndall phenomenon. JCS lxi 148.
Pierce 1910 Coagulation of substrate and enzyme action. JACS xxxii 1517.
Piper 1912 "Elektrophysiologie Menschlicher Muskeln." Berlin.
Planck 1890 Formula for diffusion potential. APC xl 561.
Plesch 1909 Hemodynamics. ZEP vi.
Pohl 1891 Chloroform is concentrated by erythrocytes, lecithin, cholesterin and oil. AEP xxviii 210.
Polanyi 1911 Blood is acid in starvation. BZ xxxiv 192.
Porges 1905 Agglutinin or heat transforms bacteria into suspnesoids. ZB xl 133.
1911 Acidosis. WKW.
1913 CO_2 tension and respiration. BZ liv 182.
—— & Neubauer 1907 Lecithin precipitated by Ba, Sr, $Ca^{\cdot}$ and Mg like suspensoid. BZ vii 152.
Port 1910 Effect of ions on hemolysis by saponin. Resistance greater the less P (lecithin?) erythrocytes contain. DAKM xcix 259.
Porter 1911 Is rennin pepsin? JP xlii 389.
1914 Adsorption of trypsin by serum alb. BJ viii 50.
Portier 1910 Δ of marine vertebrates. JPP xii 202.
Posnjak 1912 Swelling pressure of gelatine. KCB iii 417.
Poulton 1915 Alv. CO_2 below 2.5% anticipates coma. PRS (M) vii 171; JP l (p. i).
Pototsky 1902 Diuresis and NaCl starvation. AGP xci 584.
Prenant 1910 Mitosis. JAP xlvi 511.
Pribram 1911 Lipoids precipitated by anesthetics. AGP cxxxvii 350.
Price 1914 Ultramicroscopy of plant cells. AB xxviii 601.
Prideaux 1911 PH of phosphate mixtures. BJ vi 122.

Pringsheim 1912 "Die Reizbewegungen der Pflanzer."
Prowazek 1903 Phagocytosis of echinoderm egg. ZAP ii 385.
1907 Formation of plasma membrane. BC xxvii 734.
Pugliese 1906 Effect of salts on smooth muscle. AIB xlvi 371.
Putter 1904 Toxicity of great O_2 pressure. ZAP iii 363.
1911 Vergleichende Physiologie. Jena, Fischer.
Pulst 1902 Cu non toxic to penicillium. JWB xxxvii 205.
Quagliariello 1910 Saline cathartics. BZ xxvii 517.
1912a Change of PH during heat coagulation of proteins. BZ xliv 157.
1912b PH normal in fever. BZ xliv 162.
Quincke 1888a Plasma membrane fat. APk xxxv 580; SKPA xxxiv 791.
1888b Adsorption of colloids. APk xxxv 582.
1902 Surface tension of sols. APk ix 969.
Quinton 1900 Δ of marine animals. CRA cxxxi 952.
1904 Change of Δ of fish. CRB lvii 470.
Raben 1905 N and Si in sea water. Wissensch. Meeresunts. Kiel, viii 81-286.
Radziszewski 1883 Compounds that emit light on oxidation at low temperature. BDCG x 597.
Raehlmann 1906 Ultramicroscopy of protein. AGP cxii 128.
Ramsay 1894 Diffusion pressure of gases. PM xxxviii 206; ZPkC xv 518.
Ramsden 1904 Haptogen membranes. PRS lxxii 156; ZPkC xlvii 343.
Ransom 1901 Cholesterin protects erythrocytes from saponin. DMW 194.
Raoult 1901 Formula for osmotic pressure. AUG xiii 173.
Raulin 1870 Salt solution for plants. CRA lxx 634.
Rayleigh 1879 Surface tension measurements. PRS xxix 71.
1899 Tyndall phenomenon and color of sky. PM xlvii 375.
Rayleigh, G. B. 1915 Oxidasis and respiration. JBC xxii 99.
Reid 1900 Ligature of lymphatics does not decrease absorption by gut. PT (B) cxcii 231.
1901 Negative osmose of diaphragm of live gut wall. JP xxvi 436.
1904 Osmotic pressure of purified protein = 0. JP xxxi 438.
Rehfuss 1915 Achylia gastrica. AJMS cl 72.
——, Bergein & Hawk 1914 Residuum in empty stomach. JAMA lxiii 11.
—— & Hawk 1914 Constant acidity of gastric juice. JAMA lxiii 2088.
Reicher 1908 Anesthesia leads to pathological acid production. ZKM v 235.
Reinders 1913 Abnormal partition coefficient and hydrolysis. KZ xiii 96.
Rhumbler 1898 Contractile vacuole. AE vii 103.
1905 Ameboid motion. ZWZ lxxxiii 1.
1910 Phagocytosis. AE xxx 194.
Ribbert 1913 Path of excretion of hemoglobin by kidney. ZP xxiv 244.
Richards, A. 1914a X-rays and cell division. BB xxvii.
1914b X-radiation of enzymes. AJP xxxv 224.
1915a X-radiation. AJP xxxvi 400.
1915b Physiology of radioactivity. S xlii 287.
Richards, T. 1897 n/10 calomel electrode. ZPkC xxiv 39.
1906 Nephelometer. ACJ xxxv 510.
1915 Atomic compressibility. JACS xxxvii 1643.
—— & Wells 1904 Nephelometer. ACJ.
Richardson, Nicol & Parnell 1904 Diffusion of H_2 (as H) through Pt. PM (6) viii 1.
Riddle & Spohn 1912 Change in composition and permeability. S xxxv 462.
Rigg 1916 Salts in kelp. S xliii 602.
Ringer, S. 1880-3 Effect of salts on heart. JP iii 380, iv 29, 222.
1886 Effect of salts on muscle. JP vii 291.
—— & Phear 1895 Effect of salts on tadpoles. JP xvii 423.
—— & Gainsbury 1890 Salts and coagulation of blood. JP xi 369.
Ringer, W. 1910 Uric acid and phosphates. ZPC lxvii 332.

1916 Pepsin anodic even in acid. $H^{\cdot}$ acts on substrate. VKAW xviii 738.
—— & Van Trigt 1912 PH and ptyalin. ZPC lxxxii 484.
Roāf 1910 Osmotic pressure of proteins reduced by salts. QJEP iii 75, 171.
1912a Hemolysis. QJEP v 131.
1912b Marine organisms. JP xliii 449.
1913 O_2 tension of tissues. PRS (B) lxxxvi 215.
1914 Muscle contraction. PRS (b) lxxxviii 139.
Robertson 1906 Strychnine antitoxic to Na_2SO_4. JBC i 507.
1907 Dissociation of proteins. JPC xi 437, 542.
1910 Dissociation of acid and alkali albumins. JPC xiv 377, 528.
1911 Cell division. AE xxxii 308.
1912 Oocytin from serum and sperm. JBC xii.
Rodier 1899 Δ of marine vertebrates. TLA 103.
Rohde 1906 Haptogen membranes. AP (4) xix 135.
Rohonyi 1912 Protein binds $H^{\cdot}$ and Cl'. BZ xliv 165.
Rolly 1912 PH in disease. MMW.
1913 PH in disease. ZN xlvii 617.
Rona 1901 Cl' in blood. BZ xxix 501.
—— & Doeblin 1910 Permeability of erythrocytes to sugar. BZ xxxi 215.
—— & Gyoergy 1913 Diffusible Na and CO_2 in blood. BZ xlviii 278.
1913 When PH is less than 4.8 serum protein binds Cl'. CO_2 increases diffusible alkali in serum. $Na^{\cdot}$, $K^{\cdot}$, Cl' and free CO_2 in blood. BZ lvi 416.
—— & Michaelis 1909a Charcoal adsorbs sugar. BZ xvi 489.
1909b Permeability of erythrocytes. BZ xvi 60, xviii 375.
1909c $Ca^{\cdot\cdot}$ in milk. BZ xxi 114.
—— & Wilenko 1914 PH and use of sugar by heart. BZ lix 173.
—— & Takahashi 1910 Permeability of erythrocytes to sugar. BZ xxx 99.
1911 $Ca^{\cdot\cdot}$ in blood. BZ xxxi 336.
1913 All $Na^{\cdot}$ and $K^{\cdot}$ in serum are diffusible. ⅓ Ca is bound. BZ xlix 370.
—— & Tosh 1914 Urethane displaces adsorbed dextrose from animal charcoal. BZ lxiv 288.
Röntgen & Schneider 1886 Adsorption of ions. Negative adsorption. AP xxix 165.
Rosa 1913 Weston cell = 1.01827 at 20°. BBS ix 493.
Rosenthaler 1910 Synthesis of amygdalin with emulsin. BZ xxviii 408.
Ross 1911 "Induced Cell Division and Cancer." Blakistons.
Rossi 1909 "La Reatione delle urine nelle forme febrille." Naples.
Rost 1911 Nucleus permeable to dyes. AGP cxxxvii 359.
Rowntree, Marriott & Levy 1915 PH of dialysate with indicator. JHHB xxvi 114.
Rubner 1913 Fermentation by yeast same in 2.5% and in 20% sugar. Synthesis of glycogen. AAP (Sup.) 240.
Rudolphi 1895 Dilution law for strong electrolytes. ZPkC xvii 385.
Ruhland 1908 Diffusion through artificial lipoid membranes and permeability to acids. JWB xlvi 1.
1912 Ultrafiltration and vital staining. BDBG xxx 139.
1913 Colloids and cells. KZ xii 113.
Ryffel 1909 Lactic acid and acidosis. JP xxxix (p. xxix).
Rywosch 1907 Erythrocytes of ox and sheep containing most cholesterin are most resistant to saponine and least to hypotonicity. AGP cxvi 229.
Salamonson 1916 Psychogalvanic reflex. VKAW x 997.
Salge 1911 Δ of newborn. KZ i 126, ii 347.
1912 PH of newborn. KZ iv 171.
Salm 1906-8 Indicators. ZPkC lvii 471, lxvii 83.

Salzmann 1913 Individual factors in narcosis. AEP lxx 233.
Samec 1912 Swelling of starch: Li, Mg, Ca, Na, K, Sr, Ba. KCB iii 1, 123.
Savchenko & Aristovsky 1912 PH and phagocytosis. ASB xvii 128.
Scatchard & Bagert 1916 Dinitrobenzoylene-urea PH 6-8. S xliii 722.
Schade 1910 Gall and bladder stones due to lack of protection colloids. KCB i 375.
1910 Lyotrope series and association of H_2O color of sols. KZ vii 26.
1913 Tendons swell in acid and shrink in alkali. ZEP xiv 1.
—— & Boden 1913 Colloidal solutions of uric acid. ZPC lxxxiii 347; lxxxvi 238.
Schafer 1902 Swelling of frog's embryos. AE xiv 306.
Schaffnit 1911 Freezing out of colloids ZAP xii 223.
Schaffer 1888 Rate of diffusion. ZC ii 390.
Scheurlen 1896 NaCl increases toxicity of phenol. AEP xxxvii 84.
—— & Spiro 1897 NaCl increases toxicity of phenol. MMW 81.
Schimkewitsch 1902 Amitosis. BC xxii 605.
Schlaepper 1906 Oxidation in cells. AGP cxiv 301.
Schloss 1912 Water retention favored by Na and inhibited by Ca. ZK iii 441.
Schmidt, C. 1909 Conversion table: millivolts and $H^{\cdot}$. UCPP iii 101.
Schmidt, G. 1910 Adsorption saturation. ZPkC lxxiv 689, lxxvii 641.
Schmidt-Neilsen 1909 Change of Δ of fish. NVS 20.
1909-10 Adsorption of colloids irreversible. ZPC lxviii 317; lx 426.
Schneider 1913 Alveolar CO_2 tension after descent. AJP xxxii 295.
Schönbien 1863 Catalysis. JPC lxxxix 323.
Schorr 1912 Protein most easily precipitated by albumin at isoelectric point. BZ xxxviii 424.
Schryver 1910 Photosynthesis of formaldehyde. PRS (B) lxxxii 226.
Schucking 1903 Fertilization membrane preformed. AGP xcvii 85.
Schütt 1904 Haptogen membranes. APm (4) xiii 712.
Schütz 1885 Peptic digestion proportional to square root of pepsin concentration. ZPC ix 577.
Schulemann 1912 Dispersion and vital staining of Goldmann pyrrol cells. APm ccl 252.
Schulz 1912 Δ of newborn. ZK iii 251, 405.
Schulze 1884 Schulze's rule (valence). JPkC xxvii 320.
Schwarz 1907 Ions restoring excitability of sugar muscle. AGP cxvii 161.
1911 Tetanus increases osmotic pressure of muscle. BZ xxxvii 34.
—— & Lemberger 1911 Effect of $H^{\cdot}$ on blood vessels. AGP cxli 149.
Schwartz 1913 Skin current of frog. ZP xxvi 734.
1915 Frog's skin current. AGP clxii 162, 547.
Schücking 1902 Marine invertebrates semipermeable. AAP 533.
Schwyzer 1914 PH and blood corpuscles. BZ lx 297, 306.
Scott, F. 1908 Regulation of respiration. JP xxxvii 301.
1915 Permeability of erythrocytes to protein. JP l 128.
1916 Permeability of blood vessels. JP l 157.
Scott, G. 1913a Osmotic equilibrium of fish. BB xxv 121.
1913b Osmotic equilibrium of fish. ANYA xxiii 1.
Scott, R. 1916 PH and dissociation of oxyhemoglobin. JLCM i 608.
Scudder 1914 "Conductivity and Ionization Constant of Organic Compounds." N. Y.
Sellards 1914 Acidosis in nephritis. JHHB xxv 141.
Senter 1903-10 Enzymes. ZPkC xliv 257, li 673, 681, 701, lii 511.
Shaklee & Meltzer 1909 Shaking enzymes. AJP xxv 81.
Shattuck & Dudgeon 1912 Effect of drying bacteria. PRS (B) lxxxv 127.
Sheppard 1914 "Photochemistry." Longmans.
Shields 1893 Hydrolysis of KCN. ZPkC xii 167.

Shorter 1909 Haptogen membranes. PM (6) xvii 560.
—— & Ellingworth 1916 Soap and not OH' emulsifies. PRS (A) xcii 231.
Shull 1913 Permeability of hulls of Xanthium seeds like plasma membrane. BG lvi 169.
Siebeck 1912 Permeability of kidney cells. AGP cxlviii 443.
1913 Permeability of muscle to KCl. AGP cl 316.
Siedentopf & Zsigmondy 1903 Ultramicroscope. APk x 1.
Simon 1910 Protein-salt compounds. ZPC lxvi 70.
Slator 1903 Catalyser may alter type of reaction. ZPkC xlv 513.
Snyder 1908 Temp. coef. of vital processes. AJP xxii 309.
Sollman 1903 Selective action of kidney. AJP ix 425, 454.
1904 Fundulus egg permeable to water (?). AJP xii 112.
—— & Pitcher 1911 Asphyxia stimulates vasomotor center. AJP xxix 100.
Sörensen, S. P. L. 1909 $H^{\cdot}$ and enzymes. BZ xxi 130, xxii 352.
1912 PH methods. EP xii 393.
—— & Palitsch 1910 Alphanaptholphthalein. BZ xxiv 381.
1913 PH of sea water. BZ li 307.
Souza 1911 Reduction of thermolability of trypsin. JP xliii 374.
Spaeth 1913 Effect of salts on chromotaphores. JEZ xv 527.
Spiro 1904 Acids and alkalis increase swelling of gelatine. BCPP v 276.
—— & Bruns 1898 NaCl increases toxicity of phenol. AEPP xli 355.
—— & Henderson, L. 1908 Model of blood alkalescence. $Na.Alb = H.Alb + NaHCO_3$. BZ xv 114.
Spring 1896 Deltas formed by precipitation of mud by salts of sea water. NR.
Stadler & Kleemann 1911 Adsorption of NH by erythrocytes. BZ xxxvi 301, 321.
Starling 1899 Diffusion from tissues into blood. Osmotic pressure of proteins. JP xxiv 317.
1915 "Principles of Human Physiology." Phila.
Stempell 1914 Contractile vacuole. ZJ xxxiv 437.
Stern 1912 Osmotic pressure of concentrated solution. ZPC lxxxi 441.
Stewart 1897 Electric conductivity of erythrocytes. ZP xi 332.
1899 Increase in conductivity of erythrocytes. Method of determining their volume. JP xxiv 211, 356.
1901 In hemolysis salts come out before hemoglobin. JP xxvi 470.
1909 Hemolysis. JPET i 49.
Stieglitz 1908 Catalysis. ACJ xxxix 62.
Stoklasa 1908 Ash of azobacter = K_2HPO_4. ZBk xxi.
1916 Significance of K in plants. BZ lxxiii 107.
Strachan & Chu 1914 Transference no. of I. JACS xxxvi 810.
Straub 1904 Adsorption of veratrin by heart of aplysia. AF i 65.
Straub 1912 Salts produce current of injury of heart. ZB lviii.
1913 Effect of alkaloids on muscle and heart. ZP xxvi 990.
Stübel 1911 Fluorescence of tissues in ultraviolet light. AGP cxlii 1.
1914 Ultramicroscopy of blood coagulation. AGP clvi 361.
Sumner 1905 Osmose and fish. BBF xxv 53.
1907 Osmose and fish. AJP xix 61.
1911 Objective study of sight in fishes. JEZ x 409.
Svedberg 1909 Colloidal sulphur. ZPkC lxvii 249.
1909 No boundary between solutions and sols. ZPkC lxvii 105-251.
1910 Brownian movement. KZ i.
Swellengrebel 1905 Plasmolysis of bacteria. ZB xiv 374.
Szili 1906 PH of fetal blood. Dogs killed by infusion of HCl at PH = 6.05. AGP cxv 72, 82.
—— & Friedenthal 1903 Buffers for Ringer's fluid. AAP 550.
Szücs 1910 Permeability. SWA cxix 737.
1911 Effect of electric current on permeability. JWB lii 326.

1912 $Al^{\cdots}$ increases permeability of spirogyra. JWB lii 85.
1913 $Al^{\cdots}$ increases viscosity of spirogyra H_2O_2 kills. JWB lii 269.
Szyszkowski 1908 Adsorption on foam. ZPkC lxiv 387.
Tamman 1888 Isosmotic solutions. APk xxxiv.
1892 Permeability of Traube's membranes. ZPkC x 255.
Tanaka 1911 Acids on castor bean lipase. JCET v 25.
1912 PH and lipase. JCET v 125.
Tangl 1906 PH of stomach. AGP cxv 64.
—— & Farkas 1904 Respiration of fish eggs. AGP civ 624.
Tashiro 1913 CO_2 production by nerve. AJP xxxii 107, 137.
—— & Adams 1914 Anesthesia and CO_2 by nerve. JZP-CB i 450.
Taylor 1906 Synthesis of fat by lipase. JBC ii 87.
1907 Synthesis of protein by trypsin. JBC iii 87.
1915 "The Chemistry of Colloids." Longmans.
Teague & Buxton 1906 Charge of yeast reversed by ions. ZPkC lvii 76.
1907 Immune bodies cathodic. JEM ix 254.
1907 Dispersion of colloids. ZPkC lx 479.
Temple 1915 Salting out of alcohol. Thesis. U. of Minn.
Tennent 1910 PH and Mendelian dominance. CIWP (No. 132) 117.
Thiel & Strohecker 1914 Hydration of CO_2 and dissociation of H_2CO_3. BDCG xlvii 945.
Thiselton-Dyer 1899 Seeds live at —250°. PRS lxv 361.
Thomas 1915 Anesthetics increase viscosity of lecithin suspensions. JBC xxiii 359.
Thornton 1910 Naturally cathodic plant cells. PRS (B) lxxxii 638.
Thunberg 1911 Autoxydation of lipoids. SAP xxiv 90.
Tigerstedt 1910 Handbuch der Physiologischen Methodik. Leipsic.
Titoff 1910 Adsorption and solid solution. ZPkC lxxiv 641.
Toda & Taguchi 1913 Δ of frog's urine. ZPC lxxxvii 371.
Torrey 1912 O_2 and polarity. U. Cal. Pub. (Z) ix 249.
Tower 1896 KCl lessens diffusion potential. ZPkC.
Traube, I. Effect of ions on density of solution. ZAC iii 11.
1904 Stalagmometer. Surface tension of stomach less than blood. AGP cv 541, 559.
1908a Anesthetics inhibit hemolysis. Hemoglobin is hemolytic. BZ x 371.
1908b Surf. Ten. norm. urine greater than blood. Less in nephritis. AGP cxxiii 419.
1908c Surface tension and hemolysis. BZ xvi 371.
1909 Parthenogenesis. BZ xvi 182.
1910 Haftdruck theory. BZ xxiv 323.
1911 Haftdruck theory. AGP cxl 109.
1912 Viscostagometer. BZ xlii 500.
1913a Adsorption and solution. AGP cliii 273.
1913b Catalysis. AGP cliii 309.
1913c Many lipoid-insoluble dyes penetrate cells. BZ liv 305.
1915 Narcosis. AGP clx 501.
—— & Köhler 1915 Solutions and swelling opposed to gelation and shrinking. JZPS ii 427.
—— & Marusowa 1915 Swelling of seeds. JZPB ii 370.
Traube, M. 1867 Precipitation membranes. AAP 87, 609.
Trauty 1905 Chemiluminescencz. ZPkC liii 1.
Trendelenburg 1912 Effect of salts on smooth muscle. AEP lxix 79.
Trillat 1904 Oxidase action of Mn + colloid. CRA cxxxviii 274.
Tröndle 1910 Light increases the permeability of plant cells. JWB xlviii 171.
True 1914 Toxicity of Aq. dist. AJB i 255.
1915 Conversion table for sea water. S xlii 732.

Tschagowetz 1907 Bioelectric currents. ZB l 247.
Turner 1915 "Molecular Association." Longmans.
Tyndall 1869 Tyndall phenomenon. PM (4) xxxvii 384.
Tyrode 1910 Saline purgatives. Perfusion fluid. AIPd xx 20S.
Underhill 1916 Acidosis causes sugar retention. PSEB xiii 111.
Urano 1908a Accommodation of nerves to anisotonic solutions. ZB xxxii 459.
1908b Δ of frog's muscle press juice = .62°. ZB (NF) xxxii 212.
1908c Permeability of muscle. ZB (NF) xxxiii 483.
Usher & Priestly 1906-11 Photosynthesis. PRS (B) lxxvii 369; lxxx 318; lxxxiv 101.
Usui 1912 Part. coef. erythrocytes same after extracting lipoids. ZPC lxxxi 173.
Van Bemmeln 1899 Spontaneous alteration of vapor pressure of gels. ZAC xx.
1900 $K^{\cdot}$ of K_2SO_4 adsorbed leaving H_2SO_4 in solution. ZAP xxiii 111, 321.
Van Calcar & Loby de Bruyn 1904 Precipitation of dissolved crystalloids by centrifugal force. RTCP xxiii 218.
Van der Laan 1915 Δ of blood same as milk. BZ lxxi 289.
Van Slyke & Cullen 1914 Formula for urease action. JBC xix 141.
——, Cullen & Stillman 1915 CO_2 in blood increased after meals. PZEB xii 184.
—— & Zacharias 1914 PH = 8.7 for urease-urea combination. PH = 7 for urease decomposition. JBC xix 181.
Van Rysselberghe 1899 Turgor regulation. Plant cells permeable to KNO_3. MARB lviii 1.
1901 Temperature coefficient of rate of plasmolysis. BARB 173.
Van't Hoff 1887 Formula for osmotic pressure. ZPkC i 488.
Vernon 1904 Antitrypsin. JP xxxi 346.
1911 Indophenol oxidase. JP xlii 402.
1912a Anesthetics below a certain high concentration do not affect indophenol oxidase. JP xlv 197.
1912b Toxicity of anesthetics to oxidase. BZ xlvii 375.
1913 Autocatalysis of trypsinogen. JP xlvii 325.
1914 Oxidation and lipoids. BZ lx 202.
Verworn 1909 Theory of narcosis. DMW (No. 37).
1913 Irritability. New Haven. Yale Univ. Press.
Verzar 1912a Perfused muscle continues high rate of respiration minutes after tetanus. JP xliv 243.
1912b Thermo-current of nerve. AGP cxliii 252.
1912c Lack of oxygen and tissue respiration. JP xlv 39.
Voigt 1914 Kupfer cells of liver take up colloidal silver. DMW (No. 10).
Von Brücke 1908 Non contracting muscle may produce action current. AGP cxxiv 215.
Von Dungern & Coca 1907 Hemolysis. MMW (No. 47).
1908a Hemolysis. BKW 7.
1908b Hemolysis. MMW xlv 105.
1908c Hemolysis. BZ xii 407.
Von Elissafoff 1912 Effect of ions on electroendosmose. ZPkC lxxix 385.
—— & Freundlich 1912 Electric polarization of glass. ZPkC lxix 385.
Von Erlanger 1897 Surface movements of nematode eggs. BC xvii 152, 339.
Von Fürth & Lenk 1911 Muscle rigor. BZ xxxiii 341.
Von Halban 1909 Temperature coefficient of chemical reactions. ZPkC lxvii 169.
Von Jaksch 1890 Effect of diet on gast. acidity in children. ZKM xxiv 565.

Von Schroeder 1903 Viscosity of gelatine increased by acids and alkalis. ZPkC xlv 88.

Von Veimarn 1910 Colloid state of crystalloids. Submicrons in gelatine. KZ vi 277, vii.

Von Wyss 1908 Reduction of Cl in blood by feeding NaBr and its effect on nervous system. AEP lix 186.

Von Zeynek & Von Bernd 1910 Stimulation impossible with 100,000 alterations per sec. AGP cxxxvii 1.

Wachendorff 1911 Respiration of infusion. ZAP xiii 101.

Wächter 1905 Permeability to sugar. JWB xli 165.

Waentig & Steche PH for catalase. ZPC lxxii 226, lxxvi 177.

1912 Action of catalase on H_2O_2 is adsorption catalysis. ZPC lxxix 446.

Wagner 1890 Lyotrope series and viscosity. ZPkC v 31.

Wagner, H. 1914 Action of light on chlorophyll. PRS (B) lxxxvii 386.

Walbum 1913 Protein bleaches many indicators. BZ xlviii 291; l 346.

Walden 1892 Permeability of Traube's membranes. ZPkC x 699.

Walker, J. 1910 "Introduction to Physical Chemistry." 6th Ed. Macmillan.

—— & Cormack 1900 Dissociation constants of weak acids. JCS lxxvii 13.

—— & Marshall 1913 Hemoglobin positive. JACS xxxv 820.

Waller 1900 Electric effect of light on leaves. JP xxv (p. xvii).

1904 Increased conductivity of plant on stimulation. JLS (Bot.) xxxvii 32.

Wallace & Cushing 1898 Rate of diffusion and of absorption by gut. AJP i 411.

Walpole 1914 H_2 electrode. BJ viii 131.

1914 Aggregation of sols. BJ viii 170.

Walters 1912 Reaction products on trypsin. JBC xii 43.

Warburg, E. 1899 Formula for alternating current. APk lxvii 497.

Warburg, O. 1908 Oxidation of egg increases on fertilization. ZPC lvii 1.

1909a Oxidation of erythrocytes. ZPC lix 112.

1909b Cleavage of egg may be prevented by OH' which increases oxidation. ZPC lx 443.

1909c CO_2 analysis. ZPC lxi 261.

1910a Oxidation in egg not dependent on cleavage. ZPC lxvi 305.

1910b Oxidation and anesthetics. ZPC lxix 452.

1911a Cell respiration and structure. MMW lviii 289.

1911b Oxidation of cells. ZPC lxx 413.

1911c Oxidation and anesthetics. ZPC lxxi 479.

1912a No respiration in ground erythrocytes. AGP cxlv 277.

1912b Fermentation and oxidation. MMW (No. 47).

1912c HCN and oxidation. ZPC lxxvi 331.

1913 Respiration of liver granules. AGP cliv 599.

1914 Blood charcoal oxidizes oxalic acid. AGP clv 547.

1915 Egg respiration. AGP clx 324.

—— & Meyerhof 1912 Cell respiration and structure. AGP cxlviii 295.

1914 Oxidation of lecithin by iron. ZPC lxxxv 412.

—— & Wiesel 1912 Action of anesthetics on anaerobic processes and enzymes. AGP cxliv 465.

Warner 1914 Formaldehyde from chlorophyll. PRS (B) lxxvii 378.

Washburn 1909 Lyotrope series and Δ. JACS xxxi 322.

1915 "Principles of Physical Chemistry." N. Y. McGraw-Hill.

—— & Bell 1913 Apparatus for elec. conductivity. JACS xxxv 177.

Wasteneys 1916 Oxidation in egg reduced by stopping development. JBC xxiv 281.

Webster 1902 Absorption of liquids by tissues. Decennial Pub. Univ. of Chicago x 105.

Weinland 1894 Effect of salts on cilia. AGP lviii 105.

Weizsäcker 1912 Heart beat in KCN. AGP cxlvii 135.

Wessely 1903 Absorption of subcutaneous injections. AEP xlix 412.

Whatmough 1902 Surface tension measurement. ZPkC xxxix 126.

White 1911 Viscosimeter. BZ xxxvii 481.

Whitney 1908 Dessication of rotifers. AN xlii 665.

—— & Blake 1904 Polyvalent ions and cataphoresis. Removal of Cl from albumin. Peptisation. JACS xxvi 1339.

—— & Ober 1902 Ion equivalents for precipitating sols. ZPkC xxxix 630.

Widmark 1910 Δ of fresh water animals. ZAP x 431.

1911 Thunberg-Winterstein micro-respirometer. SAP xxiv 321.

Wiedemann 1852 Cataphoresis and electroendosmose. APk lxxxvii 321.

—— & Luedeking 1885 Heat of swelling of colloids. APk xxv 145.

Wijs 1893 Dissociation of water. ZPkC xii 514.

Wilke & Meyerhof 1910 Polarization and colloid precipitation with alternating current. AGP cxxxvii.

Williams, Riche & Lusk 1912 Dynamic action of protein. JBC xii 349.

Wilsmore 1901 Lyotrope series and electrolytic solution tension. ZPkC xxxvi 91.

Windaus 1908 Reaction between saponin and cholesterin. BDCG xlii 238.

Windle 1895 Electric effects produced by blood circulating in magnetic field. JAP xxix 346.

Winkleblech 1901 Ampholytes. ZPkC xxxvi 546.

Winterstein 1897 Death rigor of muscle does not occur in O_2. AGP cxx 225.

1910 Handbuch der vergleichende Physiologie.

1912 Microrespirometer. BZ xlvi 440.

1913 Anesthetics do not lessen solubility of O_2 in oil. BZ li 143.

1914 Narcosis with alcohol increases oxidation of spinal cord. BZ lxi 81.

Woelfel 1908 Distribution of salts in hemolysis. BJ iii 146.

Wolff, F. 1908 Temp. coef. of Weston cell. BBS v 309.

Wolff, J. 1908 Oxidase action of colloidal ferric-ferrocyanide. CRA cxlvii 745.

Wood 1903-6 Affinities of feeble bases. TCS lxxxiii 568, lxxxix 1839.

1908 Amphoteric metallic hydroxides. TCS xciii 411.

Woodland 1911 Gas secretion by fish. AA xl 225.

Worm-Müller 1870 Membranes and transference numbers. PAP cxl 116.

Yasuda 1900 Acclimatization. JCS xiii 101.

1900 Turgor regulation. JCS xiii 101.

Yatsu 1905 Cytasters in enucleated eggs. JEZ ii 287.

Ylupoe 1913 Isoelectric point of casein. ZK viii 224.

Zamidzki 1903 Adsorption on foam. ZPkC xlii 612.

Zangger 1908 Membranes can act like valves. Alcohol increases permeability of yeast to enzymes (144). EP vii 99.

Zsigmondy 1906 Colloidal gold. ZPkC lvi 65.

1906 Sols without Tyndall phenomenon. ZEC xii 631.

1912 Submicrons in gelatine. KZ xi 145.

1912 "Kolloidchemie" (p. 249). ½% gelatine submicrons on cooling.

1913 Ultramicroscope. PZ xiv 975.

—— & Bachmann 1914 Immersion ultramicroscope. KZ xiv 281.

Zuelzer 1907 Addition of sea water stops contractile vacuole. Sitzb. Ges. Natf. Freunde. 90.

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Hysteresis: Danysz, Freundlich.

Immune Bodies: Biltz, Bordet, Beniasch, Bernstein and Simonds, Craw, Eisenberg and Volk, Field and Teague, Porges, Teague and Buxton.

Ions, Action of: Beneche, Eisler, R. Ellis, Forster, Frey, Gray, Grünwald, Guthrie and Ryan, Herbst, Loeb, Mathews and Brooks, Mines, Morgan and Stockard, Osterhout, Ringer and Phear, Spaeth, Von Wyss. Antagonistic Action of I.: Bernoulli, Fischer, Freund, Galeotti, Linder and Picton, Loeb, L. and Cattell, L. and Gies, Loew, Meltzer and Auer, Osterhout, Spaeth.

Ionic Speed: Bredig, Kohlrausch.

Ion-Protein Compounds: Cohnheim, Handowsky, Manabe and Matula, Pauli and Handowsky, Pfeiffer and Von Modelsky, Rohonyi, Rona and György, R. and Takahashi, Simon.

Indicators: Fowler, Bergeim and Hawk, Friedenthal, Harvey, Levy, Rowntree and Marriott, Lubs and Clark, McClendon, Michaelis and Davidsohn, Wo. Ostwald, Palitzsch, Salm, Scatchard and Bagert, Sörensen and Palitzsch, Walbum.

Inhibition: Chiari and Frolich, Gaskell, Hagglund, Hagan and Ormond, Hering, Howell and Duke, Meek and Eyster.

Intestines: Cohnheim, Hooker, Quagliariello, Rona and Neukirk, Tyrode.

Isoelectric Point: Buglia, Hardy, Kozawa, Landsteiner, Michaelis, M. and Davidsohn, M. and Rona, Schorr, Ylupoe.

Light Perception: Eldridge-Green, Hartridge, Henri.

Lymph: Heidenhain, Leathes.

Lyotropic Series: Bechhold, Gouy, Grützner, Höber, H. and Waldenberg, Hofmeister, Kölichen, Loeb and Wasteneys, McIntosh, Pauli, Schade, Washburn, Wilsmore.

Mass Action Law: Guldberg and Waage.

Micro Analysis: Hamburger, A. B. Macallum, McDonald.

Milk: Abderhalden, Burri and Nussbaumer, Davidsohn, Friedenthal.

Muscle, Smooth: Fienga, Trendelenberg, Meigs, M. and Ryan, Pugliese. Striated M.: Höber and Spaeth, Mines, Beccari, Jensen and Fischer, Katz, Meigs, Von Fürth and Lenk, Winterstein. M. Contraction: Benedict and Cathcart, Berg, Bernstein, Cohnheim and von Uexkull, Cooke, Du Bois-Reymond, Ebbecke, Embden and Kondo, Engelmann, Fletcher, F. and Brown, A. V. Hill, Höber, Macallum, McClendon, McDougall, Meigs, Overton, Pauli, Ringer, Roth, Schwarz, Straub.

Negative Osmose: Bartell, B. and Hocker, Cohnheim, Dutrochet, Flusin, Freundlich, Girard, Graham, Hamburger, Heidenhain, Reid.

Nephelometer: Richards, R. and Wells.

Nerves: Alcock and Lynch, Baeyer, Grützner, Lodholz, Lucas, Mathews.

Non-Electrolytes: Gudzent, Loeb, Moruzzi, Temple.

Optical Rotation: Pauli, Samec and Strauss.

Osmosis: Aubert, Bartell.

Osmotic Pressure: Findlay, Arrenhius, Barger, Berkley and Hartley, Clowes, Friedenthal, Hedin, G. N. Lewis, Loeb and Wasteneys, H. N. Morse, Pfeffer, Ramsay, Raoult, G. G. Scott, Stern, Sumner, Tamman, Van't Hoff. O. of Colloids: Bayliss, Biltz, Donnan, D. and Harris, Ducceschi, Duclaux, Gatin-Gruzewska, Lillie, Moore and Roaf, Reid, Roaf, Starling. O. Regulation: W. J. Dakin, Fredericq, Garrey, Henri and Lalou, Mayenburg, Mouton, Overton, Pantanelli, Quinton, Schmidt-Nielsen, G. G. Scott, Sumner, Van Rysselberghe, Yasuda.

Oxyhemoglobin: Barcroft and Camis, B. and Hill, Bohr, Donders, Douglas, Freundlich and Bjercke, Haldane and H., A. V. Hill, Hüfner, H. and Gansser, Wo. Ostwald, Scott.

Oxidation: Ehrlich, Banta, Brodie and Vogt, Drury, Fenton, Hyman, Krogh, McClendon, Mathews, Murlin and Kramer, Onaka, F. H. Scott, Thunberg, Warburg, Widmark, Winterstein, Wolff. O. and Anesthetics: Batelli and Stern, Vernon, Warburg, Winterstein. O. in Cells: Batelli and Stern, Child, Harden and McLean, Heilbrunn, Höber, Itami and Pratt, Lillie, Loeb and Wasteneys, McClendon and Mitchell, Meyerhof, Moldovan and Weinfürter, Morawitz, Schlapper, Tangl and Farkas, Tashiro, Wachendorff, Warburg, W. and Meyerhof, Wasteneys. O. Enzymes: Bunzell, H. D. Dakin, Kastle, Bach and Chodat, Engler and Wild, Herzog and Meier, Moore and Whitley, Reed, Trillat, Vernon, Warburg, W. and Meyerhof. O. and Oxygen Pressure: Bert, Demoor, Fallois, Henze, Lehmann, Loeb and Wasteneys, Mitscherlich, Pütter, Roaf, Torrey, Verzar. O. and PH: Mathews, McClendon and Mitchell, Warburg. O. in Tissues: Batelli and Stern, A. V. Hill, Verzar. O. in Crushed Cells: Batelli and Stern, Büchner and Rapp, Bunzel, Fletcher and Hopkins, Meyerhof, Warburg.

Partition Coefficient: Berthelot and Jungfleisch, Löwy, H. Meyer, Overton, Pohl, Reinders, Usui.

Permeability: Bartell, Bayliss, Bechhold, Bethe, De Vries, Endler, Harvey, Höber, Katzenellenbogen, Kite, Kuhne, L'Hermite, Loeb, Magnus, Sorgdräger and von Leeuwen, Mathews and Longfellow, Nernst, Overton, Riddle and Spohr, Rost, Schüking, Szücs, Walden. P. of Blood Vessels: Magnus, F. H. Scott. P. to Dyes: Eisler and von Portheim, Bethe, Evans and Schulemann, Höber and Kempner, Kuster, Löwe, Michaelis and Davidsohn, Schulemann, Traube. P. of Eggs: Brown, Harvey, Loeb, McClendon, Overton, Sollman. P. of Erythrocytes: Calugareanu and Henri, Constantino, Hamburger, Hedin, Höber, Kozawa, Masing, Moore and Roaf, Rona and Doblin, Rona and Takahashi, F. H. Scott, Stewart. P. of Glands: Asher and Karaulow, Siebeck. P. of Muscle: Fahr, Jacoby and Golowinski, Meigs, Overton, Siebeck, Urano. P. of Plants: Fischer, Harvey, McClendon, Merril, Osterhout, Overton, Paine, Wächter. P. of Seeds: Atkins, A. Brown, Shull.

Periodicity: Bohn, Bredig, B. and Weinmayr, F. Darwin, Küster, Liesergang, Lyon.

Phagocytosis: Hamburger, H. and de Haan, H. and Hekma, Koltzoff, L. Loeb, Prowazek, Rhumbler, Savchenko and Aristovsky, Voigt.

Potentiometer: Bovie, McClendon.

Photogenesis: Callaud and Pelletier, Coblentz, Dubois, Harvey, Radziszewski, Stubel, Trautz.

Photosynthesis: Allen, Biltz, Berthelot and Gaudechon, Bodenstein, Bunsen and Roscoe, Gibson and Titherly, Hallwachs, Hughes, Luther and Forbes, B. Moore, M. and Webster, Schryver, Sheppard, Usher and Priestley, Wagner, Warner.

Physiological or Perfusion Fluids and Cell Media: Göthlin, Herlitzka, Lewis, Locke, Raulin. PH of P.: Mines, Rona and Wilenko, Schwartz and Lemberger, Schwyzer.

Plant Movements: Bose, Pringsheim, Ewart.

Plasma Membrane: Kuster, Lepeschkin, Prowazek, Quincke.

Plasmolysis: Bartetzko, Hamburger, Osterhout, Overton, Schwellengrebel.

Psychogalvanic Reflex: Gildemeister, Salamonson.

Refraction: Pearl and Gowan, Botazzi.

Regeneration: Cary, McCallum, Moore.

Respiratory Center: Campbell, Douglass, Haldane and Hobson, D., H., Henderson and Schneider, Gasser and Loevenhart, Haldane and Priestley, Hasselbalch, Milroy, Porges, Winterstein.

Resistance: Hutchison, Wo. Ostwald, Pictet, Thistleton-Dyer.

Sea Water: Allen and Nelson, Bethe, Clarke, Dittmar, Dole, Drew, Forschhammer, Krogh, Macallum, Matthews, Palitzsch, Raben, True.

Secretion: Bainbridge, Benrath and Sacs, Bergeim, Bohr, Botazzi and Enriquez, Fitzgerald, Mendel and Thatcher, Woodland.

Solubility: Bechhold and Ziegler, Bodlander and Fitting, Gudzent, Hulett, Wm. Ostwald, Pauli and Samec, Philip, Schade, S. and Boden, Vernon, Winterstein.

Stimulation: Adrian, Bethe, Chapin, Erlanger and Garrey, A. V. Hill, Höber, Lapique, Lillie, Loeb and Ewald, McClendon, Mathews, Nernst, N. and Barratt, Von Zeynek and von Bernd Verworm, Warburg.

Stomach: Cannon, Carlson Orr and Brinkman, Davidsohn, Hess, Rehfuss, R., Bergeim and Hawk. PH of S. Contents: Boldyreff, Frankel, McClendon, Menten, Michaelis and Davidsohn, Rehfuss and Hawk, Tangl, Von Jaksch.

Surface Tension: Allen, Berczeller and Csaki, Billird and Bruyant, B. and Dieulafe, Botazzi, Colton, Cramer, Davis, Donnan and D., Duclaux, Dupré, Fanno and Mayer, Frenkel and Cluzet, Gibbs, W. J. Jones, Harkins and Humphrey, Kaufler, Kisch, W. Lewis, Macallum, McClendon, Michaelis, Morgan, Quincke, Rayleigh Traube Whatmough. S. T. of Colloids: Berczeller, Botazzi.

Suspensions: Einstein, Freundlich and Mäkelt, Perrin, Spring.

Suspensoids: Barus, Bredig, Duclaux, Svedberg, Zsigsmondy.

Spermatozoa: De Meyer, Hirokawa, Koltzoff, F. Lillie.

Swelling: Fischer, Demoor, Goodridge and Gies, Loeb, Meigs, Meyer and Cohn, Nasse, Schäper, Schloss, Traube and Marusawa. S. of Colloids: Beutner, Chiari, Ehrenberg, Fischer and Moore, F. and Sykes, Hauberisser and Schönfeld, Henderson, Palmer and Newburgh, Lewites, Ludwig, Pauli and Rona, Posnjak, Samec, Schade, Spiro, Wiedermann and Ludeking. Osmotic S.: Beutner, Bialaszewicz, Hirokawa, McClendon. Turgor: Correns, Massart.

Synthesis: Kölichen, Loew. (See Enzymes.)

Temperature Coefficient: Bernstein, Clausen, Ganter, Kanitz, Krogh, Lucas, Masing, A. G. Mayer, A. Moore, Snyder, Van Rysselberghe, Von Halban.

Thermodynamics: Bernstein, Dupré, Gibbs, Lewis and Randall, Meyerhof, Verzar.

Tropisms: Jennings, Loeb. Galvano T.: Bancroft, Cohn and Barrat, Dale, Gassner, Greely, Loeb and Budgett, Ludloff, Mendelssohn. Geo T.: Czapek, Haberlandt, Kanda, Lyon, McClendon, Nemec. Photo T.: Ewald, Engelmann, Loeb, L. and Wasteneys, Mast. Rheo. T.: Allee, A. and Tashiro, Allen, Lyon.

Tyndall Phenomenon: Picton and Linder, Rayleigh, Tyndall, Zsigmondy.

Ultrafiltration: Bechhold, Burian, Hatschek, Ruhland.

Ultramicroscopy: Bachmann, Cesana, Cohn, Donnan and Potts, Gatin-Gruzewska and Biltz, Höber, Le Blanc, Linder and Picton, Menz, Michaelis and Pincussohn, M. and Rona, Perrin, Price, Rählmann, Siedentopf and Zsigmondy, Stübel, Teague and Buxton, Von Veimarn, Zsigmondy, Z. and Bachmann.

Undercooling: Bachmetjew, Kodis, Muller-Thurgau.

Vasomotor Center: Mathison, Sollman and Pitcher.

Viscosity: Arrenhius, Wo. Ostwald, Szücs, Traube, Wagner, White. V. of Blood: Albutt, Austrian, Bayliss, Boveri, Burton-Opitz, Heubner, McCaskey. V. of Colloids: Bingham and Durham, Hatschek, Laquer and Sackur, Michaelis and Mostynski, Pauli and Samec, P. and Wagner, Thomas, Traube, Von Schröder.

Water: Armstrong, Bousfield and Lowry, Guye, Hulett, Kohlrausch and Heydweiller, Krogh, Lowe, Lowenherz, Lorenz and Boehi, Roaf, Sörensen and Palitzsch, Wijs.

Weston Cell: Rosa, Wolff.

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